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# A STUDY OF HYPERPLASIA OF THE BONE MARROW IN MAN*

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Clinical observations and studies of the peripheral blood have made important contributions to the differentiation and classification of the various types of anemia, but a cloud of mystery still obscures many of the fundamental processes which underlie the diseases of the blood-forming organs. Even when the ultimate cause of an anemia is recognized, the way in which it is produced often remains entirely unknown. The problem of the pathology of the diseases of the blood appears at first sight to be wholly one of physiology, but the trend of recent investigation indicates that in no other field are structure and function more closely interrelated, and it soon becomes apparent that the study of the normal and pathologic physiology of the blood cannot be approached without a simultaneous study of the morphology of the bone marrow. Unfortunately the structure of active human bone marrow, even under normal circumstances, is extremely complex, and in many pathologic conditions the histology is so confused as to defy direct analysis. If, therefore, the diseases of the hematopoietic system are to be satisfactorily interpreted in terms of bone marrow function it would seem worth while to pay attention to the simplest pathologic changes, and the observations to be reported in this paper are the result of a study of the early stages of hyperplasia developing in the atrophic femoral bone marrow in man.

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The fact that comparatively little is known at present about the pathology of the diseases of the blood is largely due to the imperfect and inaccurate information available as to the processes of normal blood formation and destruction, and the many opinions and hypotheses which have been hitherto put forward in this field have contributed little of significance when applied to clinical conditions. Much new light has been thrown on the subject, however, by the recent work of Sabin, Doan and Cunningham[1,2,3,4] and their observations (based on animal experiments, with occasional references to man) appear to be a starting point from which the pathology of the clinical anemias may be gradually built up. Doan recognized that it is almost impossible to determine the structure of a tissue which is as complex as normal animal bone marrow, and in order to simplify the conditions he produced an experimental hypoplasia and then studied the successive stages of the hyperplasia which developed as the marrow returned to normal. In man, however, the conditions are in some ways more favorable to the investigator than they are in the lower animals, for while the active marrow of the vertebrae and flat bones is extremely complex, the marrow of the long bones is normally, at least in greater part, fatty and hypoplastic. Man, therefore, normally provides the necessary hypoplastic and relatively simple bone marrow, and in pathologic conditions one can find the different stages of hyperplasia which correspond to those produced in animal experiments.

Hyperplasia of the bone marrow, in the sense of an increase of cellularity, is a very common type of reaction in human disease and while the more extreme forms, with complete replacement of fat by marrow cells, are usually associated with diseases primarily affecting blood formation, less extensive degrees of hyperplasia are met with in a great variety of conditions, including many acute infections. It would be unwarranted to suggest that the changes which take place in the bone marrow in different acute infections are always similar in character or that any of them necessarily represent early stages of what is found in any particular form of severe anemia, for there is plenty of evidence to indicate that there are many types of bone marrow hyperplasia. It is reasonable, however, to suppose that a study of the simpler hyperplasias of acute infections and the steps in their development from normal hypoplastic marrow may assist the subsequent analysis of more complex pathologic pictures.

The present paper deals directly with the bone marrow of a single case of typhus fever, but the main observations have been confirmed in other cases of the same disease and in other pathologic conditions. This case was selected because of the excellent state of preservation of the tissue and because it shows so many of the stages in the transition from a completely fatty bone marrow to one with a cellularity approaching that of normal human vertebral marrow in the area of a single section. Fig. 1 illustrates, with low magnification, the general character of the material. In passing from the normal to the cellular part of the section it is comparatively easy to trace the successive steps in the development of the hyperplasia. The tissue is an example of relatively pure erythropoiesis and it is fortunately in a state of vascular engorgement in which many of the blood vessels are defined by blood cells almost as clearly as they would be by an artificial injection mass.

The necropsy was performed by Dr. S. B. Wolbach in Poland in 1920, and I am indebted to him for permission to use the material from the case.

The tissue, which was from the femur, was cut in serial sections 6 microns thick, and emphasis must be laid on the fact that many of the observations recorded can only be made by a study of serial sections. In describing the tissue an attempt will be made to consider the various steps in the progressive development of the hyperplasia as nearly as possible in the sequence in which they probably occur. The anatomic nomenclature of Sabin and her collaborators will be followed.

Before proceeding to a description of the pathologic material, brief mention may be made of some of the characteristics of normal human bone marrow from the shaft of the femur. In contrast to the marrow from the epiphyses, which may be moderately cellular and active in a functional sense, the marrow of the shaft of the femur is essentially hypoplastic, inactive and almost without true marrow cells. It consists of large fat cells closely packed together, with occasional elongated, darkly staining nuclei compressed between the fat globules (Fig. 3). As shown much more clearly in the early stages of hyperplasia, these are the nuclei of endothelial cells. The nuclei of the fat cells are not at all prominent and the reticular cells described by Sabin are rare and difficult to distinguish. Another striking feature is the limited degree of vascularity. Small arteries and veins are

present, but the venous sinusoids which are so evident in normal vertebral and in many hyperplastic marrows are narrow and relatively few in number.

It is difficult to determine from the histologic evidence presented by the pathologic tissue under consideration as to what is actually the initial step in the development of hyperplasia in a fatty bone marrow, for two changes are found in areas that are otherwise entirely normal and they apparently take place almost synchronously. One of these is a proliferation of the endothelial cells situated between the fat cells. Instead of the few scattered nuclei which are found in aplastic fatty marrow (Fig. 3), there are large numbers of endothelial nuclei (Fig. 2), and instead of elongated, darkly staining nuclei with little visible protoplasm, there are large, oval, vesicular nuclei with prominent cell bodies lying closely adjacent to the fat globules (Figs. 4 and 5). Doan, Cunningham and Sabin [4] also describe this hyperplasia and hypertrophy of the endothelium.

The second early change is an increase of blood supply, as shown by the appearance of new, wide venous sinusoids, and more particularly by the opening up of collapsed vessels lying between the fat cells. By means of injections with India ink, Doan [3] was able to demonstrate that the narrow, elongated endothelial nuclei lying between the fat cells in atrophic marrow are in fact the nuclei of the endothelial walls of capillaries which are not open to the circulation and which are collapsed by the pressure of the tightly packed fat cells. Drinker [6] also found evidence suggesting the presence of the same vessels. The existence of these "intersinusoidal capillaries" was indicated by the entrance into them of particles of the injection mass, but it is still more clearly shown in the stage of vascular engorgement which is part of the earliest phase of the hyperplasia in the human tissue now under consideration. At this stage, coincident with the appearance of many venous sinusoids, the intersinusoidal capillaries become injected with blood and a vast system of blood vessels, lying between the individual fat cells, is revealed. Fig. 6 illustrates, with low magnification, this early stage in the development of hyperplasia — the large venous sinusoids and the network of intersinusoidal capillaries outlining the fat cells. Some of the fat cells are sectioned at such a level that they are almost completely surrounded by patent capillaries (Fig. 8), and others have an open capillary containing blood cells on one side, and collapsed capillaries

which may be indicated by the long, narrow nuclei of endothelial cells, on the other sides. When blood enters an intersinusoidal capillary and the lumen becomes patent, the walls of the capillary are seen to be attached to the fat cells between which they have been compressed, and as the capillary lumen becomes wider the walls of the capillary remain in contact with the fat cells while the latter become in turn compressed. The structure of the wall of the intersinusoidal capillaries is similar to that of the venous sinusoids, consisting only of a single layer of endothelium, and the fact that, with the development of hyperplasia, venous sinusoids appear where one would only expect to find collapsed capillaries in the atrophic marrow, is evidence that the venous sinusoids are merely capillaries which have become widely open to the blood stream. As Doan, Cunningham and Sabin [4] have stated: "A sinus is a patent intersinusoidal capillary and an intersinusoidal capillary is a collapsed sinus, the state of dilatation or collapse normally depending upon, or at least accompanying, the specific functional capacity shown by the endothelial cells at the moment." The system of intersinusoidal capillaries may be almost completely collapsed in fatty marrow such as that of the normal human femur, and it probably takes little part in the actual nutrition of tissue. This function is carried on by a rather limited number of "transition capillaries" (Doan) which act as intermediary communications between arterioles and venous sinusoids. Fig. 9 shows one of these vessels with a small side branch, lying between fat cells and traversing a widely dilated intersinusoidal capillary.

While the extensive network of intersinusoidal capillaries seems to play no significant part in the nutrition of the bone marrow, Sabin and her associates have shown that it has a most important function in relation to the formation of red blood corpuscles, for the primitive precursors of the erythrocytes apparently arise from the endothelium which forms its walls. This phase of the problem of hyperplasia will be taken up, however, only after the question of the anatomic relationship of the intersinusoidal capillaries to the venous sinusoids has been considered, — a relationship which bears directly on the problem of the delivery of young erythrocytes into the blood stream, and one which can be studied best in the first stage of marrow hyperplasia. The failure of early investigators to recognize this system of intersinusoidal capillaries is probably explained by the

fact that they have usually concerned themselves with the study of animal marrows and the advanced pathologic changes in man, and in such tissues the intersinusoidal capillaries become masked by the enormous numbers of true marrow cells. It is only in the first stage of hyperplasia, when the marrow consists essentially of fat cells and of an open vascular bed, that one can see the relations of the intersinusoidal capillary field to the general circulation.

Doan [3] not only discovered the intersinusoidal capillary bed but he also described the manner in which the capillaries open into the venous sinusoids by means of conical openings in the walls of the sinusoids. These openings are very easily made out in the present case, for many of the capillaries are wide open and filled with red blood cells so that their ramifications can be easily followed. The large venous sinusoids are surrounded by spherical fat cells and between two fat cells one may find a capillary, which has opened up and contains blood, leading toward a venous sinusoid into which it opens by a conical aperture. The sides of the opening are formed by the endothelium of the sinusoid being carried over the convex surfaces of the fat cells so that it becomes continuous with the endothelium of the capillary. The "mouth" of the capillary, where the vessel widens out to enter the sinusoid, frequently contains a number of red blood corpuscles which appear to be about to enter the sinusoid. The upper and lower borders of the conical openings are often defined by the nucleus of an endothelial cell which lies in the horizontal plane, and in favorable places one can make out, by focusing, that the endothelium of the sinusoid bends outward in the direction of the capillary. These openings are illustrated in Figs. 7 and 10. Fig. 11 is a drawing of two openings from intersinusoidal capillaries, one on either side of a fat cell, into a venous sinusoid, and Figs. 12 and 13 are photographs, with high magnification, of each of the openings. These illustrations indicate the character of the openings with unusual clearness, especially when it is considered that they show only a single plane. Convincing evidence, as far as this can be derived from the histology, of the fact that the intersinusoidal capillaries connect with the venous sinusoids by means of conical openings in the walls of the sinusoids, is only to be obtained by focusing and by the examination of serial sections. The study of serial sections also shows that there are innumerable anastomoses between the intersinusoidal capillaries and that the openings into the venous sinusoids

are extremely numerous. In many places the openings appear to come between each pair of fat cells. The mesh of the capillary network is so complex that it unquestionably results in the formation of anastomoses between the venous sinusoids, but whether this intersinusoidal capillary bed is also directly connected with the arterioles is somewhat more uncertain. Large open capillary spaces are often found in close association with arterioles but no openings between the two have been definitely demonstrated. Histologic evidence on this point might well be unobtainable, while the finding by Doan of India ink particles, injected into the arterial circulation, in the collapsed intersinusoidal capillaries suggests they may have reached the capillaries from arterioles, although it is perhaps more probable that the granules entered the capillaries from the venous sinusoids. In the animals injected by Doan, however, the intersinusoidal capillaries were closed and the pressure in the venous sinusoids must have been low. The study of tissue, like that of the present case, in which there are areas with open capillaries and areas with closed capillaries suggests some control over the capillary bed such as is known to exist in other organs. This control over the opening and closing of the capillaries is considered by many to be regulated by the tone of the precapillary arterioles. The venous sinusoids of the bone marrow are formed by a single layer of endothelium and they are without muscular or elastic tissue. It is difficult to see, therefore, how they could regulate a flow of blood out into the capillary bed. Possibly some specific stimulus, acting directly on the cells of the intersinusoidal capillaries or on the arterioles determines their opening as well as their functional state.

Thus far only the earliest phases in the development of hyperplasia of the bone marrow have been described, and it has been shown that in the part of the tissue in which there is least deviation from the normal, the changes consist of an increased vascularization which results largely from the opening of the vast network of intersinusoidal capillaries, and the hypertrophy and hyperplasia of the endothelium of these capillaries.

The second stage in the development of bone marrow hyperplasia is characterized by the appearance of the true marrow cells from which the mature blood cells are derived. As has already been stated, the tissue under consideration shows an unusually pure erythropoiesis and no attention will be paid to the question of leucocyte formation.

The smallest cell groups in which there are only a few very primitive cells may be taken as representing the earliest step toward blood formation. In this tissue there are many areas consisting of endothelial cells and one or more definite megaloblasts, and the striking feature of these cell groups is that the megaloblasts are either attached to the endothelium which forms the intersinusoidal capillaries or they are free, within the intersinusoidal capillaries. Sabin [1] observed the formation of megaloblasts from the capillary endothelium of the living chick blastoderm and watched the megaloblasts drop off the endothelium into the lumen of the capillary. Subsequently she and her associates [4] showed that red cell formation takes place in a similar manner in adult birds and mammals. The histologic evidence derived from the study of early bone marrow hyperplasia in adult man, as illustrated by the present case, is entirely in harmony with the conception that the primitive cells of the erythrocyte series are derived from the endothelium forming the intersinusoidal capillaries, that they separate off into the lumina of these capillaries and develop to maturity within these endothelial-lined spaces. According to this view of erythropoiesis the red blood cells are formed within endothelial-lined spaces which are directly connected with the venous sinusoids and thus within the vascular system. Fig. 14 illustrates the hypertrophy of the endothelium of an intersinusoidal capillary, such as has already been seen in Figs. 4 and 5, and in addition it shows three very early cells of the erythrocyte series, probably megaloblasts, in close association with the endothelium. Fig. 15 is a photograph of a space between four fat cells, such as has been found in the stage of vascularization to be lined by the endothelium of intersinusoidal capillaries, filled by five cells of which three are probably to be classed as endothelial cells and two as megaloblasts, one of the latter being in mitosis. In Fig. 16 there is an endothelial cell, out of focus, lying adjacent to the lower fat cell, and there are two definite megaloblasts in a similar relation to the upper fat cell on the left. At this earliest stage of cellular proliferation, areas may be found in which the fat cells are entirely separated by cords of large cuboidal cells which resemble megaloblasts more than endothelial cells, but some of which are probably erythroblasts. Sabin believes that the megaloblastic stage is usually of short duration and that these cells quickly divide and become what she terms erythroblasts. Fig. 17 is a drawing to illustrate such

an area. Below is an endothelial cell filling the space between two fat cells, and the other spaces which represent intersinusoidal capillaries are largely filled by rows of megaloblasts, erythroblasts and a few more mature cells of the erythrocyte series. Fig. 20 is a photograph of the same area under higher magnification. The spaces between the fat cells, which during the stage of vascular engorgement were seen to consist of capillaries lined with flat endothelium, are in this later stage filled with columns and clumps of cells which are frequently in a state of active proliferation, as shown by the number of mitoses. Double columns of cells may also appear, indicating that the formation of megaloblasts is taking place from the endothelium on both sides of the capillary, and the capillary lumen is usually completely occluded by the cell growth. Where the section cuts the plane between the lower aspect of one fat globule and the upper aspect of the globule below it, one gets, not a column of cells, but a more or less extensive island of primitive cells of the erythrocyte series. At this stage, when active proliferation is in progress, the flat endothelium with its long narrow nuclei is much less apparent than under normal conditions and it seems to be largely replaced by the bigger cuboidal cells which line the capillary spaces. In the areas of early cellular hyperplasia the venous sinusoids are numerous, large and prominent, and the most striking areas of cellular proliferation are usually found in close relation to a venous sinusoid. This is not in harmony with the observation of Doan [4] who found in experimental animals that erythropoiesis was most marked in places in which the circulation was relatively inactive. Not infrequently a row or group of megaloblasts is situated along the outside of a venous sinusoid, between the sinus and an adjacent fat cell; the endothelium of the sinusoid, however, retains its usual flat character with long thin nuclei. It is most probable that the megaloblasts arise from endothelium covering the fat cell rather than from the endothelium of the venous sinusoid which in the case under consideration shows no evidence of taking on any erythropoietic function.

The next stage in the development of the hyperplasia of the marrow is that in which cells of more mature type than the megaloblast and erythroblast make their appearance. Again the process can be best analyzed in the smaller cell groups. After the megaloblasts have become detached from the endothelium of the intersinusoidal

capillaries, they may divide, as shown by mitotic figures, and go through the process of maturation, so that erythroblasts, normoblasts and extruded normoblastic nuclei may be found free in the capillary spaces. Mature erythrocytes may also be present, but it is, of course, impossible to determine whether these cells have developed in the intersinusoidal capillaries or whether they have been brought in from the general circulation. The fact that in the earliest stages of megaloblastic hyperplasia the intersinusoidal capillaries may be occluded by endothelial cells and megaloblasts, and contain very few erythrocytes, suggests that the erythrocytes which reappear in the capillaries with the normoblasts have actually been formed locally. The degree of maturity of the cells varies from field to field and normoblasts are often found grouped together in considerable numbers. These clumps of normoblasts may be found in the center of the capillary space, surrounded by more primitive cells, and they are also likely to lie in close relation to a venous sinusoid. Fig. 18 illustrates this phase of the process. It shows a somewhat larger space between fat cells, filled with a clump of cells containing several erythroblasts, many normoblasts with typical pyknotic nuclei and a few erythrocytes. Reasoning by analogy it may be assumed that this space is a dilated intersinusoidal capillary and the conception is borne out by the presence of the endothelial cell, with elongated nucleus, which lies closely adherent to the upper fat cell.

Up to this point the process of hyperplasia, with the development of cells of the erythrocyte series from the endothelium of the intersinusoidal capillaries and within these capillary spaces, has been comparatively easy to trace, but from now on it becomes more confusing. The cellular areas grow larger and they appear as complex masses of cells without definite structure. All the cell types thus far observed are present and new ones enter into the picture. Endothelial cells, still a part of the capillary wall, may become phagocytes of red blood cells; clasmatocytes with ingested normoblasts or erythrocytes are free in the cell mass; leucocytes of various types have made their appearance; and megalokaryocytes may also be found. Although these larger areas of hyperplasia are without doubt the seat of active erythropoiesis, they may contain comparatively few mature red cells, and these are often grouped near the venous sinusoids, as if they were ready to slip into the circulation. Fig. 19 illustrates a cellular area situated near a large venous sinusoid (on right). The

elongated nucleus of the flat endothelium of the sinusoid may be compared with vesicular nuclei of the hypertrophied endothelial cells in the angle between the two fat cells above and on the left. The latter, in close relation to the fat cell, undoubtedly belong to the endothelium lining the now greatly distended intersinusoidal capillary, and they represent the type of active endothelium from which megaloblasts originate. Free in the cell group are megaloblasts (several near the sinusoid in the upper right corner), erythroblasts, normoblasts, erythrocytes, clasmatocytes which have ingested red blood cells, and a few myelocytes and leucocytes. Fig. 22 is a drawing of a similar group of cells also near two venous sinusoids (upper right and lower left). It is impossible to illustrate these areas satisfactorily with photographs as many of the cells are indistinct at any one level of focusing. The endothelial border of this space is indicated by the flat endothelium along the upper middle fat cell and the hypertrophied endothelial cell lying next to the upper left fat cell. In the center, just below the large sinusoid, is a group of large early cells of the erythrocyte series — megaloblasts and erythroblasts. The shapes of these cells suggest that they were closely adherent to one another and that they have shrunk apart in the process of fixation. The same relationship is seen in the four megaloblasts which lie along the right middle fat cell. This tendency for early megaloblasts to remain closely attached to one another, like the component cells of a tissue, is extremely common and they are often found in rows or double columns. As they develop they seem to lose this adhesiveness and become separate, independent cells, the process being analogous to what Key [5] has described as taking place in the maturation of erythrocytes. Just below the center of the field in Fig. 22 is a megaloblast in mitosis. Fig. 23 is a drawing of a larger cellular area and one which is approximately the size of the cell areas in normal vertebral marrow. Evidences of the endothelium bordering the cell group may be found along the fat cells and the general cytology is similar to that in the smaller cellular areas but it has become complicated through the entrance of more cells of the leucocyte series. It remains, however, much less confused than the picture of normal active marrow as the number of leucocytes is relatively small, the process continuing to be, even in these most hyperplastic areas, one of comparatively pure erythropoiesis.

The bone marrow is enclosed in a rigid container, and cellular

hyperplasia with its associated vascular engorgement can only take place at the expense of something which filled the marrow cavity during the state of aplasia. Fat tissue is peculiarly adapted to being the complementary substance, for fat cells can give up their charge of fat rapidly and can become infinitely smaller without loss of function. Compression, at least within wide limits, does not injure them, for they retain their ability to take up fat again and thus fill any space that results from subsequent retrogression of the hyperplasia. Between marrow cells and fat cells there exists a remarkable reciprocal relationship, in which the former appear to dominate while the latter serve the subsidiary function of filling in any space not occupied by active myeloid tissue. Evidence of this relationship is seen in many experimental conditions in which marrow hyperplasia or aplasia, accompanied by a decrease or increase of fat, arise with extraordinary rapidity. When the cellular areas increase in size there is a simultaneous diminution in the amount of fat. Gradual compression of the fat cells produces a decrease in their size before they disappear completely, and in a very hyperplastic marrow the few remaining fat cells are usually much smaller than the fat cells in an aplastic marrow. In the tissue now under consideration the most highly cellular parts contain about twenty fat cells per high dry field as against approximately thirty per field in the aplastic areas and the individual fat cells are definitely smaller. In a highly cellular marrow, such as the typical marrow of pernicious anemia, the fat often seems to have been completely displaced, but it is certain that the fat cells continue to survive, for as soon as a remission of the disease sets in the hyperplasia of the marrow cells retrogresses and fat is again deposited in the fat cells. The manner in which the bone marrow can alternately take up and give up fat indicates that the fat cells are as constant a part of its structure as the blood vessels and the supporting framework of reticulum. When marrow hyperplasia recedes and the myeloid cells disappear there is not only a new deposition of fat in the fat cells but there is a complete, and often an extraordinarily rapid return to the structure of typical aplastic marrow.

In the highly cellular areas of the marrow, illustrated by Figs. 19, 22 and 23, the venous sinusoids are large, numerous and engorged with blood. They appear to be surrounded by an intact wall of endothelium and there is little to suggest the openings of the inter-

sinusoidal capillaries that are so clearly made out in the areas which show the very earliest stage of hyperplasia. The only possible indication of these openings, indeed, is to be found in the occasional small clumps of mature erythrocytes situated just at the edge of the sinusoid and which may mark points of entrance into the vessel. It is, however, not remarkable that the openings into the venous sinusoids are thus obscured, for the whole structure of the tissue has been completely altered. In the first stage of hyperplasia these openings connected the venous sinusoids with delicate intersinusoidal capillaries, some of which were collapsed and others, more easily distinguished, were filled with mature red blood corpuscles. This was followed by the development of cells of the erythrocyte series within the capillaries, the compression of fat cells and the formation of large cell masses within the intersinusoidal capillaries. In this late stage of hyperplasia the intersinusoidal capillaries have become wide cell beds and their individual limits have become indistinguishable just as a series of brooks which flow through a field lose their identity when the spring floods cause them to swell until they merge and convert the field into a great lake.

In spite of the fact that in a highly cellular marrow it is impossible to observe definite openings between the intracapillary cell areas which form so much of the tissue and the venous sinusoids, the study of the development of hyperplasia certainly indicates that such openings exist and that through them the mature erythrocytes enter the circulation. The process by which the cells pass from their position in the intersinusoidal capillaries into the venous sinusoids is not, however, clear. Histologic evidence shows that the more immature cells are often at the periphery of the capillary and either adherent to the endothelium or adherent to one another so as to form groups or columns, while the more mature cells occupy a central position in the cell mass. It is possible that the latter cells, which have lost their adhesive qualities, are gradually forced towards the venous sinusoid by the pressure of cell growth in the sense of Drinker,[6] and it is also possible that a true circulation through the venous sinusoids, such as appears to be present in the earliest stage of hyperplasia, persists and washes out the mature cells into the veins.

While the tissue which has been described is an example of relatively pure erythropoietic hyperplasia, it also shows a considerable

development of megalokaryocytes. Little is known about the origin of these cells but the fact that in the early phases of hyperplasia they are often found closely adherent to fat cells, in the position occupied by the endothelium of the intersinusoid capillaries, suggests that they, as well as megaloblasts, may arise from endothelium. Fig. 21 is a photograph of a megalokaryocyte in such a position.

## Summary and Conclusions

In an attempt to throw light on the pathology of the bone marrow and thus on the fundamental factors which underlie the diseases of the hematopoietic system, the histology of the femoral marrow from a case of typhus fever has been described. This tissue was selected because it is an example of almost pure erythropoiesis and because it shows the various steps in the development from an atrophic to a relatively hyperplastic marrow within the area of a single section. The first changes consist of the appearance of large venous sinusoids, the opening to the circulation of the extensive network of intersinusoidal capillaries, and the hypertrophy and hyperplasia of the endothelium lining the capillaries. At this phase the openings from the intersinusoidal capillaries into the venous sinusoids can be easily detected. In a later stage the precursors of the erythrocytes appear inside the intersinusoidal capillaries, and the histologic picture is consistent with the concept that they are derived from the endothelium of the intersinusoidal capillaries. The earliest islands of marrow cells are composed of a few megaloblasts attached to endothelial cells or free within the capillary spaces. Larger cell islands usually contain more mature types of the erythrocyte series (erythroblasts and normoblasts) and there is a general tendency for the immature cells to be adherent to one another at the periphery of the group and for the mature cells to be free and independent in the center. As the cellular areas increase in size the picture becomes complicated by the appearance of other cell types, and in this advanced stage of hyperplasia the evidence that the erythrocytes develop within capillaries which are in direct communication with the venous sinusoids becomes much more obscure. There are, however, indications that the intracapillary formation of erythrocytes persists even in highly cellular marrows, and this relation suggests the general method by which young red blood cells enter the circulation.

The histology of the type of bone marrow hyperplasia which has been described can be readily analyzed on the basis of the work of Sabin, Doan and Cunningham, and this study of human tissue is offered in support of their conceptions.

I am greatly indebted to Miss Lillian M. Leavitt for the preparation of the serial sections of bone marrow, and to Miss E. Piotti for her beautiful and accurate drawings.

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## DESCRIPTION OF PLATES

### PLATE 91

FIG. 1. General character of material described. Aplastic marrow above; marked hyperplasia below; and intermediary stages in development of hyperplasia in the center. × 50.

FIG. 2. Drawing to illustrate hypertrophy and hyperplasia of endothelium of intersinusoidal capillaries lying between two venous sinusoids. × 500.

FIG. 3. Drawing of normal bone marrow from human femur, showing elongated nuclei of collapsed intersinusoidal capillaries. × 500.

FIG. 4. Hypertrophy of endothelial cell of intersinusoidal capillary. Note relation of endothelial cell to fat cell. × 1000.

FIG. 5. Two hypertrophied endothelial cells of intersinusoidal capillary. × 1000.

### PLATE 92

FIG. 6. Earliest stage of marrow hyperplasia. Large venous sinusoids and engorgement of intersinusoidal capillaries with red blood cells. × 80.

FIG. 7. Drawing of intersinusoidal capillaries, filled with blood, opening into a venous sinusoid. × 500.

FIG. 8. Drawing of intersinusoidal capillaries, filled with blood, surrounding a fat cell. × 500.

FIG. 9. Drawing of a transitional capillary. Note hypertrophy and hyperplasia of endothelium of intersinusoidal capillaries. × 500.

FIG. 10. Drawing of intersinusoidal capillaries filled with blood and opening into a venous sinusoid. Note nuclei of endothelial cells lying on the floor of the opening. × 500.

## PLATE 93

FIG. 11. Drawing of the openings of two intersinusoidal capillaries into a venous sinusoid. Note the incurving of the endothelium of the sinusoid to meet and form the wall of the capillary. The drawing indicates the funnel-shaped character of the openings and the two following photographs confirm this appearance. × 500.

FIG. 12. Photograph of the opening shown on the left in Fig. 11. × 1500.

FIG. 13. Photograph of the opening shown on the right in Fig. 11. × 1500.

FIG. 14. Drawing of hypertrophied endothelium of intersinusoidal capillary and three megaloblasts in close relation to the endothelial cells. × 500.

FIG. 15. Endothelial cells of intersinusoidal capillary, with two megaloblasts. One megaloblast in mitosis. × 1250.

FIG. 16. Two megaloblasts in close association with fat cell (in position occupied by endothelium of intersinusoidal capillary). Endothelial cell, next to a fat cell, shown indistinctly below. × 1000.

## PLATE 94

FIG. 17. Drawing of hypertrophied endothelial cells of intersinusoidal capillaries with megaloblasts and erythroblasts developing in the capillaries. × 500.

FIG. 18. Drawing of erythroblasts and normoblasts in intersinusoidal capillary. Note endothelial cell lining the capillary spaces, above. × 500.

FIG. 19. Drawing of larger cell group near a venous sinusoid. Many normoblasts, a few leucocytes, and two clasmatocytes which have phagocytized red cells. × 500.

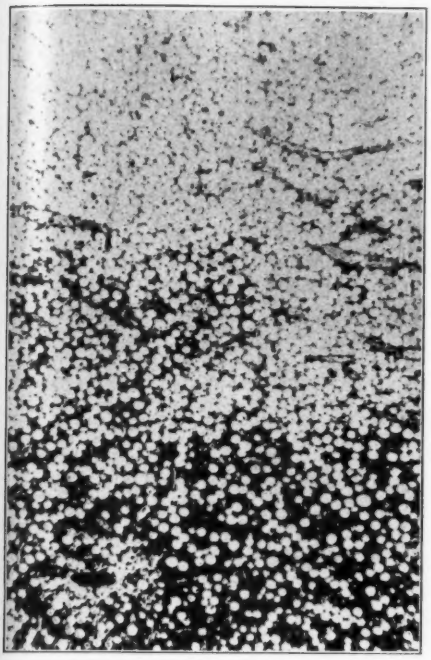
FIG. 20. Photograph of same field as Fig. 17. × 1000.

FIG. 21. Megalokaryocyte lying between fat cells, in position of intersinusoidal capillary. × 1000.

## PLATE 95

FIG. 22. Drawing of a group of cells developing between and compressing fat cells. The flat endothelial cell along the upper fat cell and the hypertrophied endothelial cell along the upper left fat cell suggest that the group of cells is inside an intersinusoidal capillary which has become greatly distended. Note megaloblasts, one of which is in mitosis, in center. × 500.

FIG. 23. Drawing of a larger group of cells. In several places elongated or vesicular nuclei of endothelial cells, lying closely attached to fat cells, suggest that the cell island is developing within a space lined with endothelium. Note further decrease in size of fat cells. × 500.



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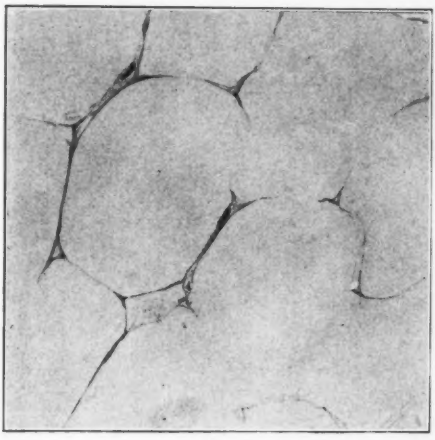

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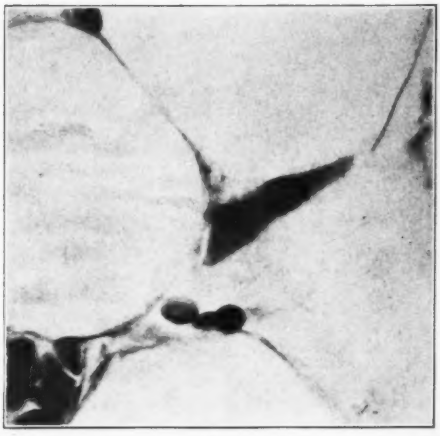

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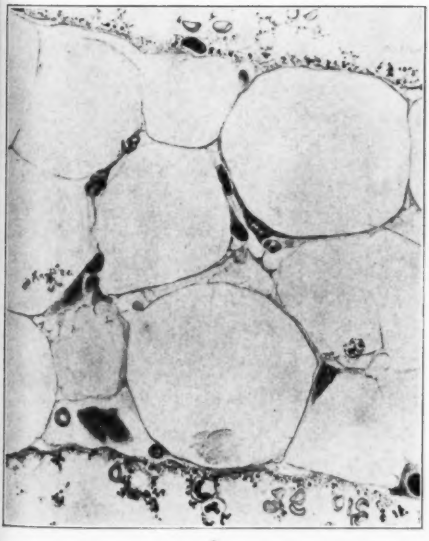

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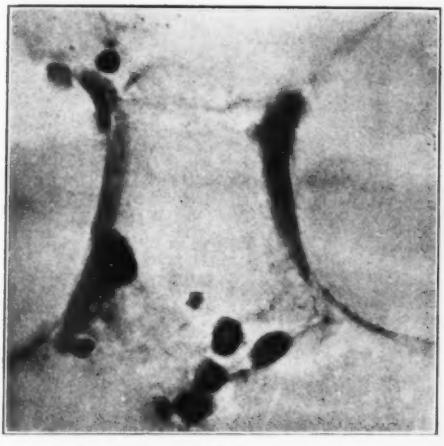

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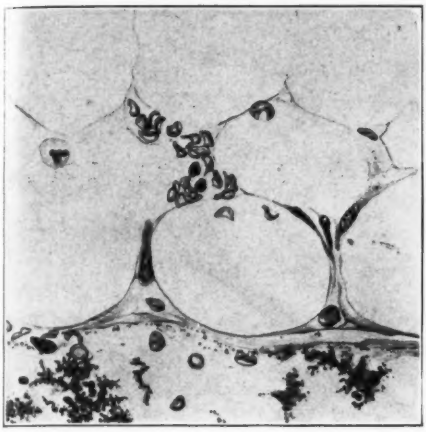
Peabody

Hyperplasia of Bone Marrow in Man



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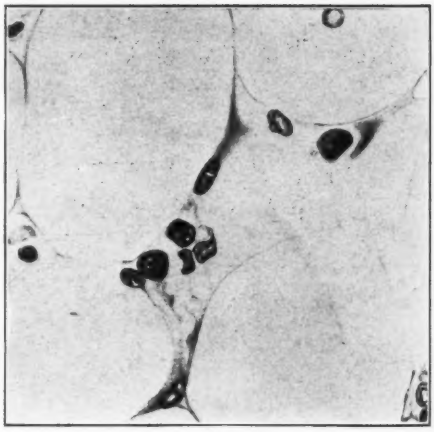

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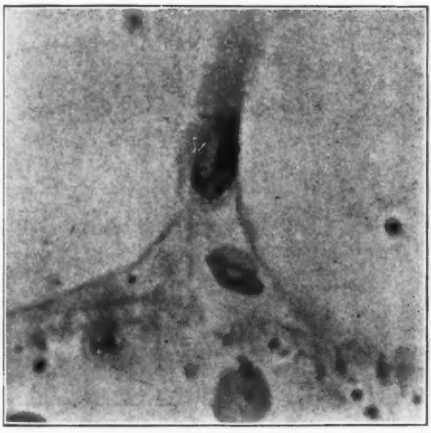

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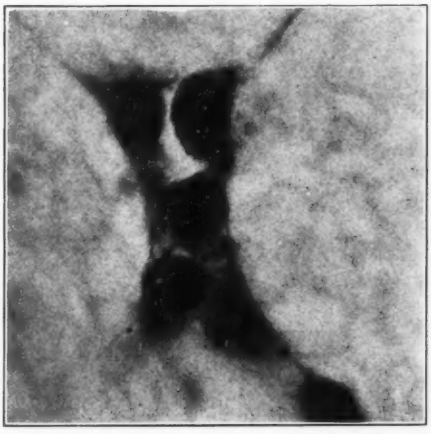

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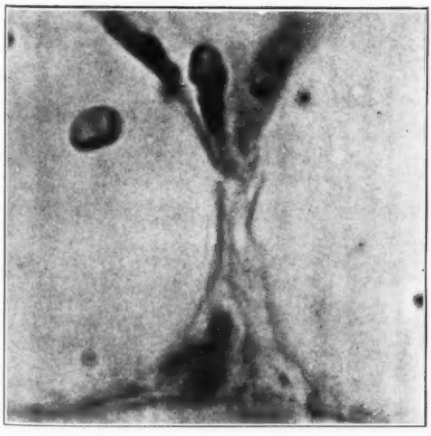

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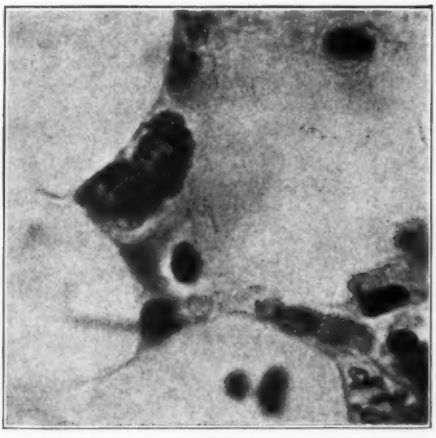

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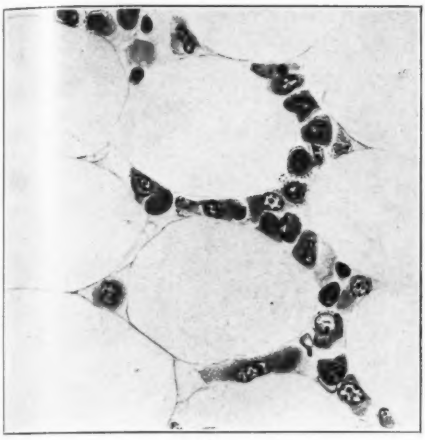

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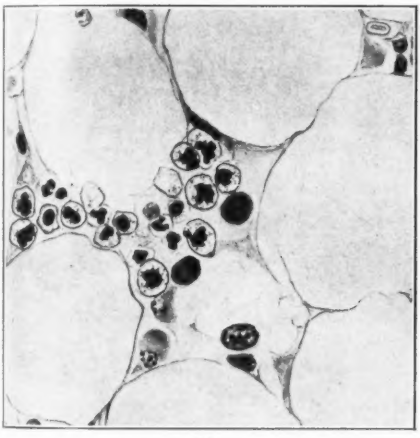

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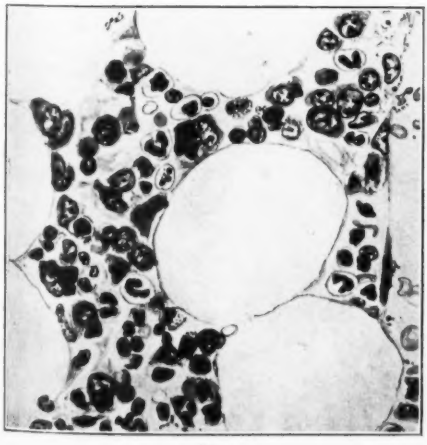

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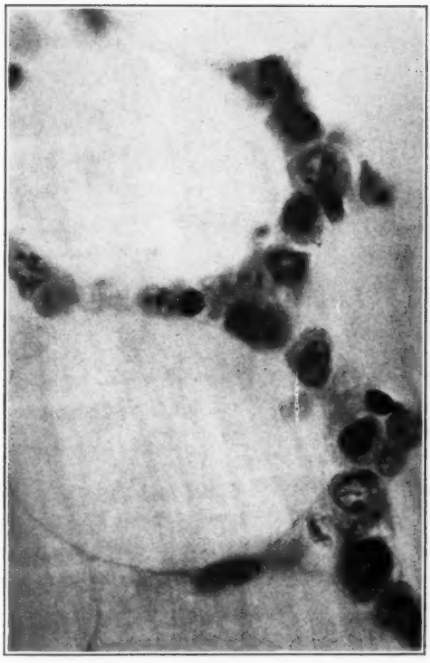

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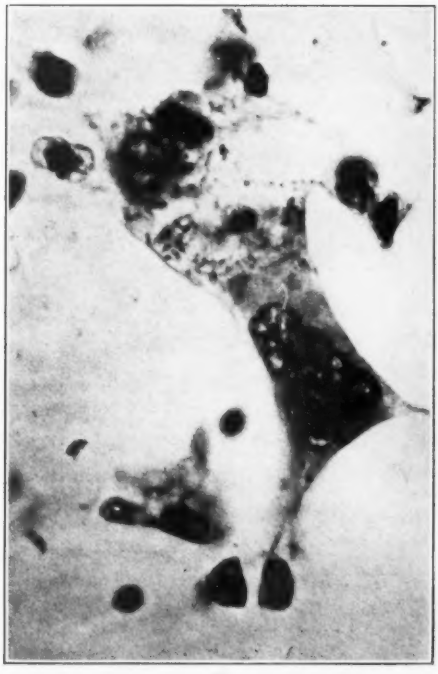

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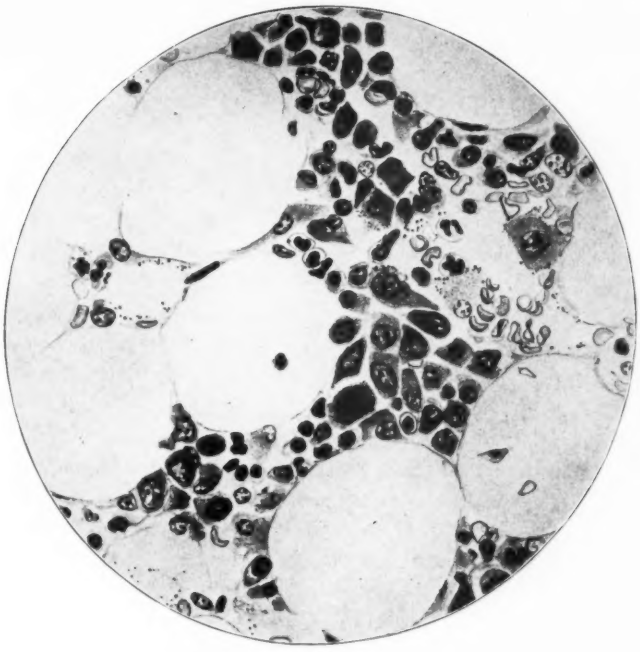
Hyperplasia of Bone Marrow in Man



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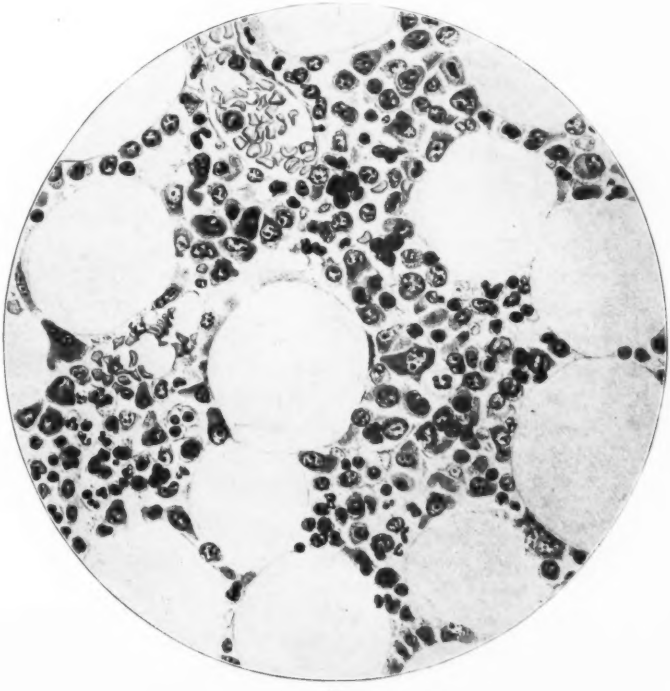

23

# FIBRIL FORMATION BY HUMAN LUTEIN CELLS*

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Several years ago I happened to observe conspicuous fibrils attached to the large cells of a well preserved and apparently normal corpus luteum. In Zenker-fixed tissue these fibrils were visible, stained bright pink with the Mallory eosin-methylene blue technic; but they were best seen after using his phosphotungstic acid hematoxylin.[1] Demonstration of these fibrils to several persons indicated that their existence was not generally known. Moreover, inquiry failed to disclose any reference to their having been already described. They were not seen again, however, in a succession of rather indifferently preserved corpora lutea studied in routine surgical tissue examinations. Neither was it possible to demonstrate them in the corpora lutea of such laboratory animals as were then immediately available, *i. e.*, rabbits and white rats. But from time to time human corpora lutea have come to hand in which fibrils similar to those first seen could be found. Therefore, it seemed worth while looking for them systematically in available surgical material.

The search has shown them with sufficient frequency to warrant a careful description of these lutein fibrils as a hitherto unnoted normal feature in the development of lutein cells. In fifteen corpora lutea the preservation was good enough so that myoglia and fibroglia were demonstrable in the sections. Lutein fibrils were distinctly seen in eleven of these fifteen corpora. Their presence was doubtful in two others and in two obviously early corpora lutea spuria they were absent entirely. In every one of five corpora lutea vera fibrils were abundant and distinct. These included one normal five months' pregnancy, three ectopic pregnancies and one post-abortion case. Of seven cases of corpora lutea spuria fibrils were well developed in one late case in which shrinkage of the corpus had begun; they were very clear but somewhat less numerous in one fully developed case, while in two others relatively few were seen, and their presence was

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doubtful in a fourth; two early cases showed no fibrils as stated above. Three cases of corpora lutea cystica are included. Fibrils were conspicuous in one, scanty in one and doubtfully present in the third. The cystic corpus in which fibrils were well developed was in the same ovary as the advanced corpus luteum spurium in which fibrils were most evident. It is indicated from these admittedly few observations that the development of fibrils is evidence of full differentiation of the lutein cell. The difficulty of obtaining even one complete series of normal human corpora lutea properly fixed will be readily appreciated.

The fibrils are to be found running in various directions over the surface or within the marginal cytoplasm. For the most part they tend to run with the long axis of the cell but they are unique among other known cell fibrils in that they frequently intersect each other so as to produce a basket-like network about the cell. Some of the fibrils are much heavier than others in the same cell. In certain preparations, also, they are larger on the average than in others. In consequence of the irregular course taken by the fibrils, not all of them appear as long straight lines even when the cell is cut longitudinally. When the cell is cut transversely or more or less obliquely, most of them are seen as short rods, sometimes curved, and as sharply stained dots. In such cases the marginal distribution of the fibrils is particularly evident. Note Figs. 7, 8, 9 and 10. It is, of course, much easier to find cells cut a little obliquely than those sectioned exactly transversely; and since the sections studied are appreciably thick (approximately 7 microns), the dots and short rods often appear to be more or less deeply embedded within a given cell. This is shown in Fig. 6. But careful focusing discloses the fact that they are in all cases strictly peripheral despite such appearances. Where the fibrils intersect there is often a more or less conspicuous thickening. The thickness of these points varies rather strikingly in different cases. In some (Fig. 5) it is very slight, while in others (Figs. 11 and 13) it is extreme. It seems possible that the age of the corpus luteum may be a factor in this. Thus, the best marked examples in the series of cases studied are to be found in a corpus luteum verum in a five months' pregnancy. In this case some cells were provided with networks of very coarse fibrils while comparatively fine fibrils in other cells were at times to be found radiating from large, irregular nodal points. These nodal foci stained some-

times as deeply as the fibrils themselves, although in other cells of the same case they did not stain blue-black with phosphotungstic acid hematoxylin but instead stained a brown-lilac color, similar to but rather deeper than the cytoplasm proper.

In 1915 Corner [2] called attention to deeply stained, often somewhat shrunken, branching cells in the corpus luteum of the sow. He designated them "additional cells of the corpus luteum, type 2" and credited Delestre with having noted them previously. Corner regards their significance as obscure, although in his later paper (1919) [3] he considers them as unaltered, migrated theca interna cells. What surely are corresponding cells are commonly present in human corpora lutea which have attained maximum development. They stain more deeply than the surrounding lutein cells, they are shrunken into an irregularly, richly branching stellate form and their nuclei are either very deeply stained (pyknosis) or exhibit karyolysis. Except for the absence of leucocytes in their vicinity, they possess all the criteria of necrotic cells and I interpret them as such. Their frequency varies in different cases as does also the degree of shrinkage. They occur singly and have not been seen in groups. My material is insufficient to warrant a definite statement as to the origin of these cells. Many of the cells in young corpora lutea spuria are stellate and have dense cytoplasm with well preserved nuclei. Whether or not these are ingrowing theca cells which later undergo necrosis I am not now prepared to say. But running out from the necrotic cells above described, deeply staining fibrils are often to be seen. Fig. 14 illustrates this feature. It is a curious fact that whereas the lutein cell fibrils are susceptible to postmortem changes in the tissue as a whole and are clearly demonstrable only in material preserved soon after operation or very early postmortem, these fibrils seem to persist in isolated, individual necrotic cells. The suggestion is advanced, therefore, that such appearances as are shown in Figs. 16 and 17 owe their origin to further shrinkage of necrotic lutein cells. This may explain the frequent occurrence of such coarse fibrillar networks as are seen in Figs. 11 and 13 in old, fully developed corpora lutea vera.

Lutein cells are generally described as ovoid or polyhedral. Excepting for the presence of marginal fibrils the human lutein cell appears from the study of this material to have no definite cell membrane, and as shown in Fig. 2 the cells not infrequently assume a

broadly stellate form. Associating these observations one is inevitably reminded of the fibroblast. But the lutein cell is always stouter, the stellate form is less evident and the fibrils vary in size in the same and in neighboring cells. Moreover, fibroglia never form the characteristic network to be found in lutein cells. The demonstration of these fibrils cannot, therefore, be cited as evidence in favor of the ancient theory of the purely connective tissue origin of lutein cells. The so-called theca interna cells or theca lutein cells of R. Meyer [4] are generally admitted to be of connective tissue rather than germ cell origin. They are often conspicuous and always to be found in groups peripheral to the mass of lutein cells and its own delicate stroma. They are much smaller than true lutein cells and stain more deeply. I have never seen fibrils in these theca cells although nearby lutein cells showed them sharply stained. My study of human corpora lutea inclines me to the view that the characteristic lutein cell represents a specialized development of zona granulosa cells which are in turn, of course, germinal in origin. It becomes a distinct type of cell.

Lutein fibrils are not easily confused with any other formation in the section. When present they are clear and unmistakable. Their demonstration requires fixation of thin slices of tissue in Zenker's fluid. Their susceptibility to postmortem change has been mentioned. It is further shown by the fact that when a thin slice (1 to 2 mm.) of a corpus luteum was fixed it gave sections in which fibrils stained brilliantly, but in another block of the same corpus 8 mm. thick, fibrils could be seen only dimly in many of the cells in central sections, staining fairly well near the outer edge. Fibrin threads in the central clot, however, stained with equal brilliancy in both preparations. Skeins of fine fibrin threads are often found between the lutein cells in early corpora lutea but there is no confusing these with the lutein fibrils herein described. They are focal, usually near groups of erythrocytes and not attached to the lutein cells. The lutein fibrils occur with equal abundance in all parts of the lutein parenchyma. Lutein fibrils differ essentially from mitochondria in the fact that they are not destroyed by acid fixatives. Moreover, the fibrils are strictly peripheral in the cell, never being found in the central cytoplasm nor arranged about the nucleus as is commonly true of mitochondria. Neither should the lutein fibrils be confused with the extracellular basket-like reticular fibers. The reticulum is com-

paratively coarse, and in Zenker-fixed material stained by Mallory's anilin blue connective tissue method the fibers of reticulum are bright blue in contrast with the clear red fibrils described in this paper.

The function of these interesting fibrils is not determined. As the blood clot in the center of the young corpus luteum is organized, the fibroblasts which in company with capillary sprouts have early made their way inward from the theca interna passing through the metamorphosing zona granulosa, multiply and give rise to a steadily increasing mass of collagen fibrils. For a considerable time, certainly until toward the close of pregnancy in the case of the corpus luteum verum, the lutein cells are provided only with a delicate stroma of reticulum. It is conceivable that the fibrils herein described are supportive in function. In common with other fibrils they may also possess a contractile quality. In all respects so far as studied they seem to belong to the general group of glia fibrils which includes the recognized neuroglia, myoglia and fibroglia. All of these are best demonstrated after chrome-sublimate fixation (Zenker) and when well fixed stain somewhat feebly with eosin but brilliantly with phosphotungstic acid hematoxylin and with Mallory's acid fuchsin stain. All are susceptible to postmortem changes which in certain instances give rise to peculiar alterations in the form of the fibrils before their actual disappearance. All are considered to lie partially or entirely within the cell body of their respective cells but are strictly marginal. Each type of fibril differs in morphologic details from the others and thereby may serve as a means of identifying the type of cell producing it.

It therefore seems proper and may be found useful to designate the fibrils of the lutein cells by an appropriate term. Lest strong objection be raised to combining Latin and Greek roots in the suggestive term *luteoglia*, I would offer as an alternative an euphonious word of purely Greek derivation, *xanthoglia*. This might be freely translated to mean the adhesive fibrils of the yellow cells or lutein cell fibrils.

W. H. Lewis [5] finds no evidence of smooth fibrils and very little of striated fibrils in cells growing in culture and concludes that such appearances as are so well known to histologists are fixation artefacts dependent upon coagulation of the contractile substance in the cytoplasm along lines of tension. Whether or not histologists

generally come to accept this view, it is important for practical purposes to recognize any structural appearance which can regularly be demonstrated in the course of cell or tissue development by the application of a standardized technic. From this standpoint it is clear that xanthoglia are special fibrillary structures demonstrable in the development of human lutein cells.

## Summary

Hitherto undescribed fibrils have been demonstrated in the marginal cytoplasm of fully developed human lutein cells. The fibrils tend to form a network enclosing each cell. The term *xanthoglia* is proposed to designate these fibrils.

## REFERENCES

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3. Corner, G. W. On the origin of the corpus luteum of the sow both from granulosa and theca interna. *Am. J. Anat.*, 1919–20, xxvi, 117.
4. Meyer, R. Über Corpus luteum-Bildung beim Menschen. *Arch. f. Gynäk*, 1911, xciii, 354.
5. Lewis, W. H. Behavior of cells in tissue cultures. General Cytology, Edited by E. V. Cowdry, Chicago, 1924, 395.

## DESCRIPTION OF PLATES

All the photomicrographs were taken at the uniform magnification of 650 diameters. The sections are approximately 7 microns thick.

### Plate 96

Fig. 1. The large, paler cells to the right are lutein cells cut approximately transversely. The peripheral fibrillar network is shown. The smaller darkly stained cells to the left are the characteristic theca interna cells and are devoid of fibrils.

Figs. 2, 3 and 4 show lutein cells cut more or less longitudinally. The course of the fibrils and their variation in size are clearly seen.

Fig. 5. The surface of a cell in the lower part of the figure has been brought into focus to show the basket-like network which encases the cell. In this instance the network is composed of very delicate fibrils.

Figs. 6, 7 and 8. Various views to show the peripheral distribution of the fibrils about the lutein cells. The cells are sectioned transversely and the fibrils, therefore, appear as dots and short black lines and form a part of the margin of the cells. The apparent inclusion of the fibrils within one of the cells

in Fig. 6 is due to the fact that the fibrils are approaching each other around one pole of the cell. The observer is looking down upon and through a truncated conoid of cytoplasm in which lies the cell nucleus and over the surface of which are spread fibrils.

PLATE 97

FIG. 9. The lutein cells have shrunken away from each other in the preparation of the material. The frequently eccentric position of the nucleus and the peripheral distribution of the branching fibrils can be seen. The photograph was taken from the margin of a corpus luteum. The small, darkly stained cells at the bottom of the figure are theca cells.

FIG. 10. One cell is almost completely surrounded by fibrils. In the center is a surface view of a cell showing a rather coarse fibrillar network.

FIG. 11. A coarse surface network with wide nodal points.

FIGS. 12 and 13. Two views of the same cell taken at different focal levels through one pole. With the nucleus out of focus in Fig. 13 the coarse fibrillar network and thickened intersections can be clearly seen.

FIG. 14. A shrinking, necrotic lutein cell with persistent fibrils.

FIGS. 15 and 16. Different focal levels of the same cell. An irregular, deeply stained body is seen in sharp focus on the surface of the cell in Fig. 16. Fibrils radiating from the body can be followed over the surface of the cell in both figures.

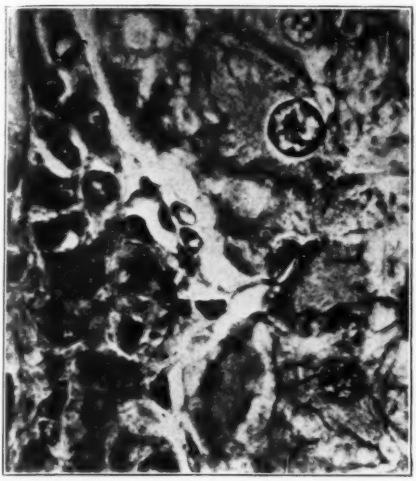
FIG. 17. The bodies seen at their intersection are sometimes more lightly stained than the fibrils.

Figs. 1, 3, 6, 7, 8, 10, 11, 12, 13, 15, 16 and 17 are from a corpus luteum in a case of a five months' intra-uterine pregnancy.

Figs. 2, 4 and 14 are from a corpus luteum in a case of tubal pregnancy.

Fig. 5 is from a corpus luteum in a case of ectopic pregnancy with secondary ovarian implantation.

Fig. 9 is from a corpus luteum spurium.



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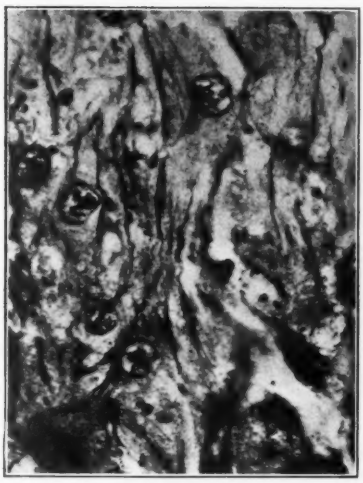

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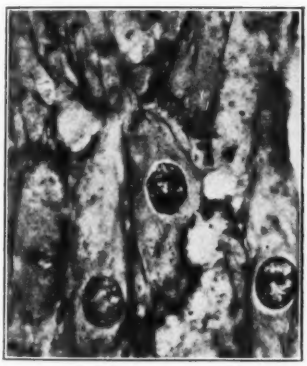

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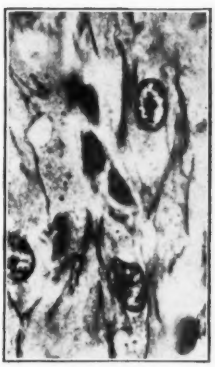

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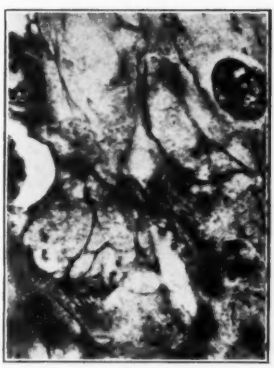

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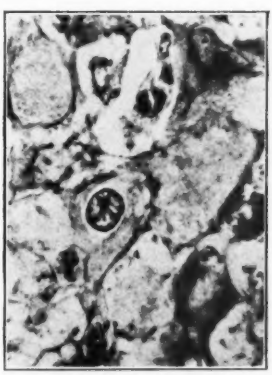

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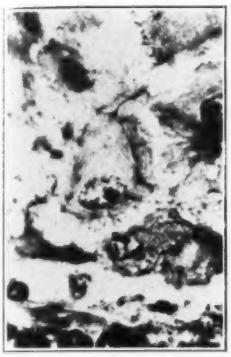

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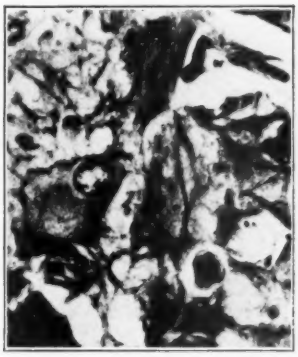

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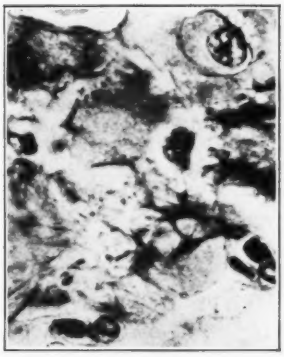

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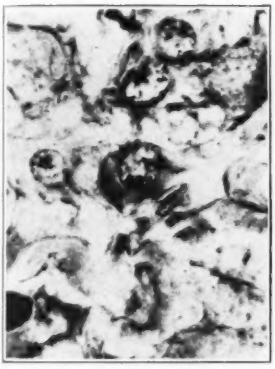

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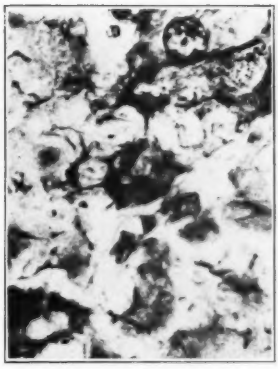

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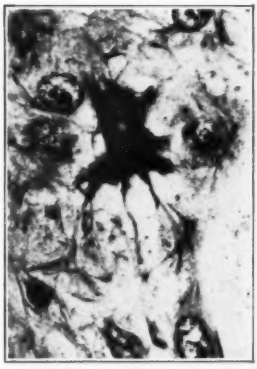

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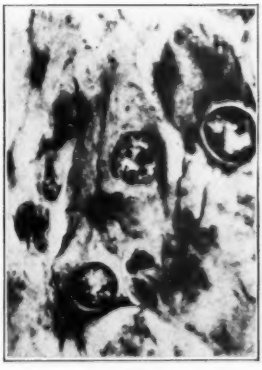

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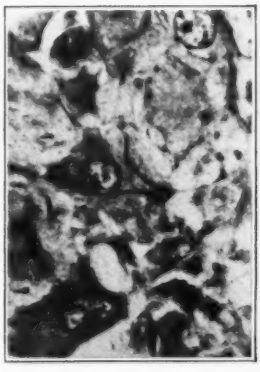

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# NECROSIS OF THE MALPIGHIAN BODIES OF THE SPLEEN *

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In 1921 Feitis,[1] under the term "*Fleckmilz*," described a condition of the spleen of which he was unable to find any report in the literature. The cut surface of the organ was studded with miliary, linseed-sized and even larger, round, oval and irregular gray-white nodules which stood out rather sharply against the reddish brown background. Most of them were small and circumscribed, but many coalesced to form larger areas. The picture closely resembled that of disseminated caseous bronchopneumonia. Microscopic examination showed that the lesions were areas of necrosis due to occlusion and thrombosis of the medium and smaller sized arteries. Feitis reported two examples of the condition, both in cases of chronic cardiovascular-renal disease with marked thickening and hyalinization of the media and intima of the smaller arteries of the spleen. Geipel [2] and Matthias [3] in 1924 each reported a case of eclampsia in which the spleen presented areas of necrosis and conformed grossly to the condition described by Feitis. In these cases fibrinous thrombi were found in the small splenic arteries. Two additional cases of arteriosclerosis with multiple necroses of the spleen were reported by Meuret [4] in 1925. In the same year Wilton [5] reported a case of acute upper respiratory infection with involvement of the frontal sinus; death followed pneumonia and pleurisy. At necropsy the pituitary gland was necrotic and the spleen was much enlarged and presented the characteristic picture of "*Fleckmilz*."

Seven cases have therefore been reported and these fall into two groups. On the one hand are those of Feitis and Meuret, in which the lesion was associated with arteriosclerosis, and on the other are those of Geipel, Matthias, and Wilton, in which the underlying condition was one of toxemia or infection. The authors who have followed Feitis have not called attention to any previously reported cases, so that it would appear that the condition is an unusual one.

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That it had previously gone unrecognized is inconceivable, since the microscopic picture presented by the condition is so striking as at once to attract attention, although the gross appearance may be deceptive and may simulate miliary tuberculosis or even simple hypertrophy of the Malpighian bodies. An additional case of multiple necrosis of the spleen, in a disease in which the process has not hitherto occurred and differing in pathogenesis from the previously reported examples, is therefore worthy of record.

## Case Report

The instance reported here was found in the spleen of Mrs. M. F., 59 years of age, admitted to the female medical service of the Michael Reese Hospital on Sept. 22, 1925. Her complaints were weakness, loss of appetite, loss of weight and numbness over the body, fingers and toes. These symptoms had begun two years previously, but she had been "run down" for eight years. Four weeks before admission she had had several blood transfusions in another hospital. Neither in that hospital nor during her stay in the Michael Reese Hospital did she receive any roentgen-ray treatment.

Physical examination revealed an elderly, emaciated woman, rather drowsy, who dozed off to sleep frequently but was easily aroused. The skin was lemon-yellow in color and the conjunctivae were slightly yellow. A few petechial spots were present over the sternum and abdominal wall and these later increased in number and became more widely distributed. The tongue was smooth, brownish in color and sore. The mucous membranes of the mouth and lips were pale. The heart, lungs and abdomen were negative. On admission the temperature was 102.8 F and ran an irregular course between 98.6 and 103.6 F, with a terminal temperature of 105.3 F. The pulse was small, regular and varied from 100 to 130 per minute. Systolic blood pressure was 100, diastolic 60. The blood Wassermann reaction was negative. Blood culture was negative. Occult blood was present on two occasions in the stools. The urine contained a trace of albumin and a few erythrocytes, leucocytes and epithelial cells. The red blood cell count on admission was 2,200,000 per cu. mm. with 60 per cent hemoglobin. The leucocyte count was 2,300 per cu. mm. with 30 per cent neutrophiles, 65 per cent small mononuclears and 5 per cent transitionals. In the stained smear poikilocytosis, anisocytosis, polychromatophilia and nucleated red

blood cells were found. On one occasion two myelocytes were present. Five days after admission the erythrocyte count was 1,850,000 per cu. mm. with 40 per cent hemoglobin. A clinical diagnosis of pernicious anemia was made. The condition became progressively worse, stupor developed and the patient died on Oct. 13, 1925, three weeks after admission.

At necropsy, performed eighteen hours after death by Doctor William Bloom, the body was that of an aged, emaciated female, with a deep lemon-yellow color to the skin and conjunctivae. Numerous petechial spots averaging the size of a common pinhead were present over the anterior surface of the chest and abdomen. The subcutaneous fat was colored a deep yellow. The heart was small and flabby, and the myocardium pale. The aorta showed small localized areas of intimal sclerosis. Patchy bronchopneumonia was present in both lower lobes. Desquamation and superficial ulceration of the lower portion of the mucosa of the esophagus and atrophy of the mucosa of the stomach were found. Numerous small, petechial hemorrhages were seen beneath the mucosa of the colon. The mesenteric lymph nodes were reddened but not enlarged. The liver was dark brown and small, measuring 22 × 15 × 18 cm. The spleen was free from adhesions, measured 9 × 5 × 3 cm., was soft, dark grayish blue in color, and had a slightly wrinkled capsule. On the cut surface the Malpighian corpuscles appeared prominent and gray, standing out against the red-brown of the surrounding tissue. The kidneys were small; the capsules stripped easily, leaving a finely granular surface. On cut section the cortex was thin, but the markings were distinct. The posterior wall of the bladder was injected and contained large, varicosed, distended veins. Two areas of necrosis each about 1.5 cm. in diameter were present about the trigone. The bone marrow of the ribs and femur was bright red and soft. The blood was thin and watery.

## Microscopic Examination

The microscopic picture of the bone marrow is one of cellular hyperplasia with numerous nucleated red cells, normoblasts and myelocytes.

The microscopic examination of the spleen yielded a surprise. The routine sections stained by hematoxylin and eosin contain numerous areas of necrosis, each one involving a Malpighian cor-

puscle. This explains their prominence in the gross specimen, and reëxamination of the formalin-fixed tissue confirmed this. In the latter, irregularly shaped small areas of cloudy gray could now be seen scattered throughout the parenchyma, resembling at first glance caseating tubercles.

The necrosis involves a Malpighian body in every instance, sometimes completely destroying its structure, but an artery can be found in practically every one, either centrally or peripherally placed (Fig. 1). The amount of necrosis is not uniform. Some are completely necrotized, others only partially, and in the latter the necrotic process affects the periphery of the corpuscles. Their character is quite uniform, differing only in the degree of necrosis, although in most of them the necrotic process is advanced and complete.

In sections stained by hematoxylin and eosin, the necrotic Malpighian bodies are violet and have a structureless appearance. The groundwork is amorphous and looks coagulated, and scattered in it are fragmented cells and nuclear granules (Fig. 2). The outer margin of the area is more or less sharp. The surrounding pulp stains a deep pink and is very cellular. The tissue spaces are filled with cells, among which are large numbers of mononuclears and pulp cells in addition to the red corpuscles. In the sinuses, immediately about the necrotic areas, are large, pale staining, spheroidal and irregularly shaped cells (Fig. 3) having a granular cytoplasm and large, eccentric nuclei, the chromatin of which is granular and possesses a reticular network. These cells fill up the sinuses, in some places completely obstructing them. They are phagocytic, for in them can be seen broken blood cells and nuclear fragments; in some, well preserved erythrocytes are present.

These macrophages arise from the endothelium of the sinuses. In the pulp and in the incompletely destroyed corpuscles they may be seen budding from the sinus endothelium into the circulation. Since the endothelium of the splenic sinuses differs from the general blood vascular endothelium in its ability to store vital dyes and is therefore considered to be an important part of the reticulo-endothelial system, these swollen phagocytic cells may be presumed to be reticulo-endothelial in origin.

While their presence is more marked around the necrotic areas, they are to be seen also within these areas; in the corpuscles which are not completely necrotized, the cells are present immediately

around the central artery and in the sinuses. Here they are not so large, the nuclei are more distinct and stain deeply, the cytoplasm is clearer, and their phagocytic properties are not so evident. Where these cells are very numerous they are crowded together and distorted, and in the necrotic areas they may be seen in all stages of disintegration. In regions farther removed from the necrotic areas, large cells the size of giant cells are present in the sinuses, which are free of blood. The cytoplasm of the larger cells is granular and their nuclei may be multilobed. Polymorphonuclear cells are conspicuous by their absence.

Mallory's aniline blue stain demonstrates very clearly the reticular connective tissue and vascular walls. These all stain a bright blue; in the areas of necrosis the connective tissue framework is coarser, enmeshing the necrotic cells. In this stain the large endothelial cells stand out rather prominently, their pale blue color forming a good contrast to the red and orange of the other cells.

In a combination stain of Weigert's iron hematoxylin and Van Gieson's connective tissue stain, the vessels and connective tissue elements are pink and the cellular elements yellow with black nuclei. In the larger vessels the intima is irregular and wavy and the media is thickened and hyaline, but nowhere is there obliteration of the lumen or thrombus formation.

Mallory's phosphotungstic acid hematoxylin demonstrates the connective tissue and reticulum in the necrotic areas as rather coarse, reddish violet strands forming an irregular, but somewhat circularly arranged framework. The individual strands are much thicker and coarser than those in the pulp. At the periphery of the necrotic follicles is a narrow, coarsely meshed zone which stains deep blue. The staining reaction is that of fibrin, but the material is unusually coarse. It does not stain by the Weigert fibrin method and is apparently swollen, partly degenerated reticulum. The arteries of the Malpighian bodies are exceptionally distinct in the phosphotungstic acid hematoxylin preparations. Their lumina are patent and there is no thrombosis in any of them.

## Discussion

In the two cases originally described by Feitis, the arteries of the spleen were thickened as part of the generalized arteriosclerotic process. In those of the Malpighian bodies, the lumen was de-

creased in diameter and was occluded by recent thrombosis. When the necrosis of the follicles was not complete, it involved the center or periarterial zone primarily, the portion farthest from the vessel being preserved. Feitis considered the process one of anemic infarction, which differed from the usual type of infarction of the spleen in that the foci of necrosis were multiple. This necrosis occurred as the result of occlusion of the smaller arteries, namely, those of the Malpighian bodies, rather than of those of larger size, as is usually the case in the more extensive infarcts which occur singly or in small numbers. Meuret noted the same changes in his two cases, which were also associated with generalized arteriosclerosis, and accepted Feitis' explanation of the necrosis.

In the two cases of eclampsia, with multiple necroses of the spleen, reported by Geipel and by Matthias, the microscopic picture in general was the same as that described by Feitis and by Meuret. Arteriosclerosis, however, was not a factor in the occlusion of the small arteries, which in these cases was also the immediate agent in the necrosis. The vessels were occluded by fresh fibrinous thrombi. The vascular endothelium had proliferated, which process and the thrombosis were ascribed to the action of a toxin. In Wilton's case of pneumonia with multiple splenic necroses, the same condition of endothelial proliferation and recent thrombosis was present, and in this case also the action of a toxin was held to be an important factor in the pathogenesis of the lesion.

In the seven previously reported cases the necrosis of the Malpighian bodies was arterial in origin. In the case here reported the arteries were not obliterated by arteriosclerosis and there was no thrombosis as the result of an acute infectious process. Furthermore, when necrosis was incomplete it spared that portion of the follicle immediately about the artery. The most striking feature of our case was the marked proliferation of the sinus endothelium. This process was generalized throughout the spleen, and was present in the red pulp as well as in the Malpighian bodies. Erythrophagocytosis by these cells was present in greater degree than one usually sees in pernicious anemia. The cellular proliferation is believed to have been a part of the anemia, and only accidentally, because of the peculiarities of the circulation within the spleen, to have been a factor in the necrosis.

Why the necrosis in this case should have begun in the peripheral

portion of the Malpighian bodies and should have been limited to the bodies requires explanation. Although the swollen macrophages are present throughout the spleen, they are most numerous in the sinusoids at the periphery of the follicles. It is only in this situation that the cells are numerous enough completely to fill the sinuses and to obstruct the circulation through them. Possibilities which present themselves are that in this situation the cells were formed in greater number, or that they were aggregated here by the peculiarities of the circulation within the spleen.

One of the last pieces of work upon which Thoma [6] was engaged before his death, and the results of which were published after his death, was a reinvestigation of the circulation of the spleen. He claimed that it is impossible to effect a complete injection of the spleen from the arterial side. If a thin injection mass with finely divided matter in suspension was used, part of the material passed through lacunae in the arterial portions of the sinusoids, the mass becoming thickened and remaining in the arterial sinusoids. If a thicker mass was used it filled the arterial sinuses but did not pass into the venous channels. By means of simultaneous arterial and venous injections with a mass of proper consistence injected under carefully controlled pressure, Thoma claimed that he was able to effect a complete injection of the sinusoidal system of the spleen. According to the results obtained by this method, the arteries follow the trabeculae and pass to the Malpighian bodies. Here each artery breaks up into a number of arterioles which traverse the follicle. At the periphery of the latter the arterioles bend back sharply upon themselves and become suddenly transformed into wide, thin-walled arterial channels which Thoma termed ampullae. Some of the arterioles pass into the pulp and here also become transformed into ampullae, but the latter are most numerous and largest immediately about the Malpighian bodies, where they form a zone of closely placed blood spaces (Fig. 4). The arterial ampullae pass over into the venous sinusoids, but the two are partly separated by a constriction which holds back the arterial injection mass (Fig. 5).

If Thoma's view of the circulation within the spleen is correct, then both the possibilities mentioned above in explanation of the aggregation of the proliferated endothelial cells in the peripheral sinuses of the follicles may have been operative. Since it is in this region that the ampullae are widest and most numerous, endothelial

proliferation would lead to a greater formation of cells here than elsewhere. In addition, the constriction between the arterial ampullae and the venous sinusoids would tend to collect within the former the cells which are formed throughout the Malpighian body and brought to the region of the constriction by the circulation. The combination of greater cellular proliferation in the ampullae and the aggregation of the cells in the latter by the circulation would cause such a filling of the peripheral vascular channels as obtained in this case. Necrosis of the Malpighian bodies due to filling of the sinusoids by proliferated cells is analogous to the focal necrosis of the spleen and liver in typhoid fever from occlusion of the sinusoids by endothelial leucocytes, as described by Mallory.[7]

## Summary

The literature contains seven cases of necrosis of the Malpighian bodies of the spleen, the first two having been reported by Feitis in 1921.

In four of these cases the underlying condition was arteriosclerosis, and necrosis was due to thickening of the walls of the arteries of the follicles with terminal thrombosis. In the remaining cases, two of eclampsia and one of pneumonia, the cause of the necrosis was also arterial occlusion, but in these the interference with the circulation was due to proliferation of the arterial endothelium and to thrombosis, both supposedly the result of the action of toxins.

An additional example of the condition, in a case of pernicious anemia, is here reported. Necrosis was limited to the Malpighian bodies, all of which were partially or completely involved in the process. The arteries of the bodies were not occluded. When the necrosis was incomplete it involved the peripheral zone and spared the tissue immediately about the artery.

The sinus endothelium was proliferated and swollen throughout the spleen, and exhibited a marked degree of erythrophagocytosis. The endothelial proliferation is believed to have been a phenomenon of the anemia.

The swollen macrophages were especially numerous in the peripheral sinuses of the Malpighian bodies, being here so crowded as to interfere with the circulation of the blood. Their aggregation in this region is held to have been due to the peculiarity of the vascular structure and of the circulation of the spleen.

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## DESCRIPTION OF PLATES

### Plate 98

Fig. 1. A group of necrotic Malpighian bodies. To the left of the middle of the field are two with a narrow non-necrotic zone about the artery. Hematoxylin and eosin. × 60.

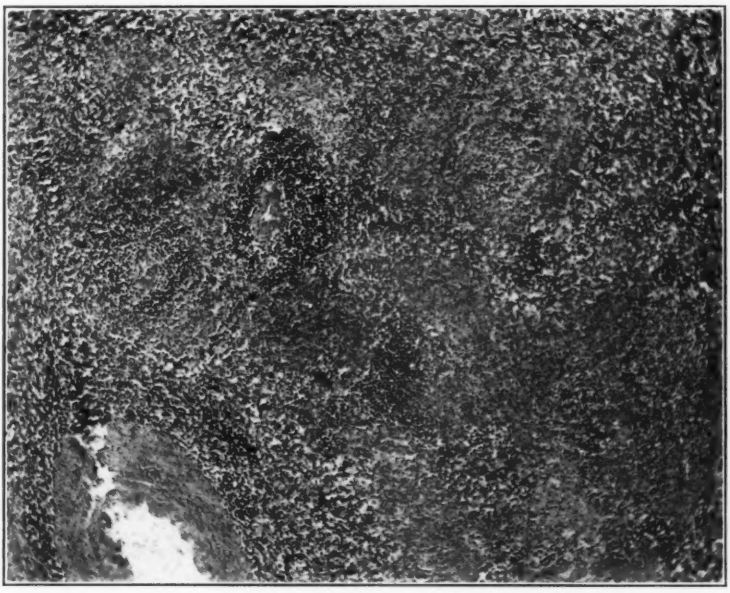
Fig. 2. Karyorrhexis in a necrotic Malpighian body. The artery is not occluded. Hematoxylin and eosin. × 325.

### Plate 99

Fig. 3. Swollen reticulo-endothelial cells within the sinusoids at the periphery of a necrotic Malpighian body. Mallory's aniline blue connective tissue stain. × 560.

Fig. 4. Ampullae about the periphery of a Malpighian body; arterial injection of spleen of dog (Thoma).

Fig. 5. Terminal arteriole, with its ampullae (black) and venous sinusoids (gray); combined venous and arterial injection of spleen of dog (Thoma).



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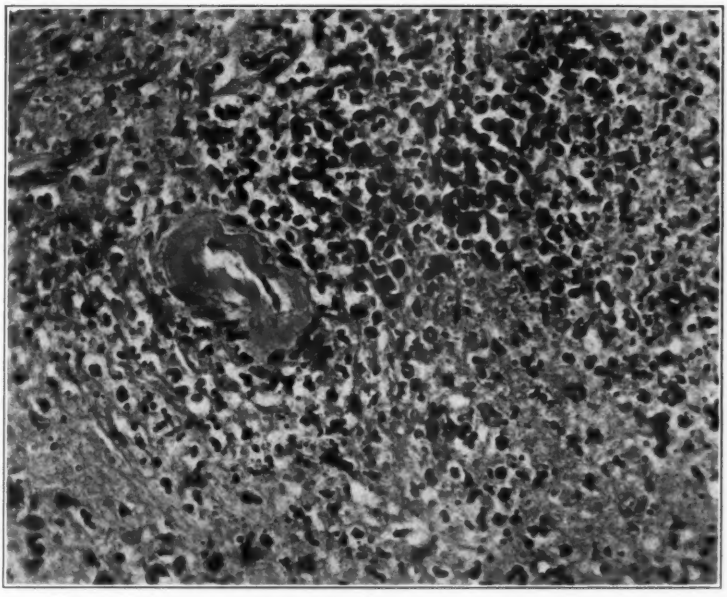

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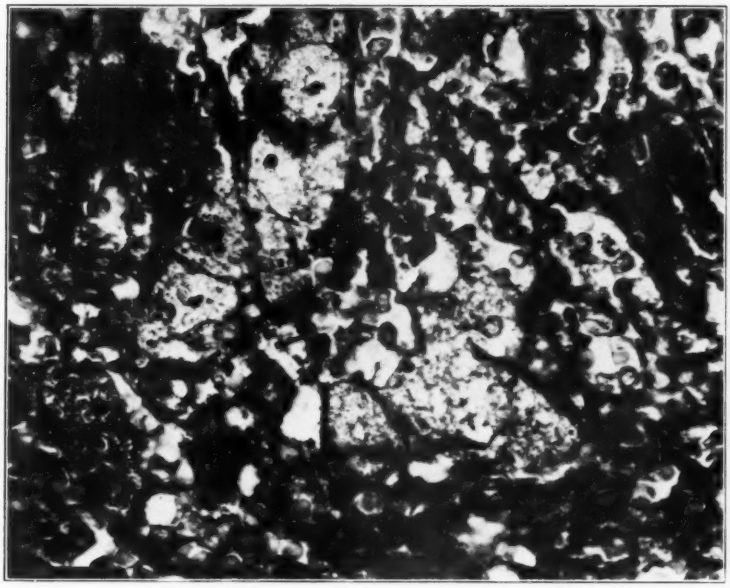
Enzer Necrosis of Malpighian Bodies of Spleen



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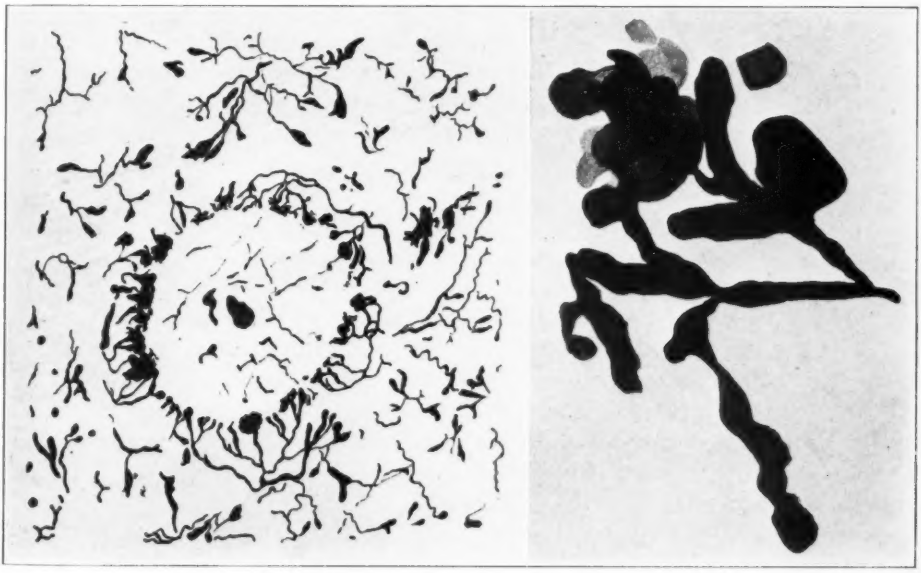

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Enzer Necrosis of Malpighian Bodies of Spleen

# HETEROTRANSPLANTATION OF CARTILAGE AND FAT TISSUE AND THE REACTION AGAINST HETEROTRANSPLANTS IN GENERAL

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In former investigations we studied heterotransplantation of skin,[1] thyroid[2] and kidney[3]; we also made some experiments in which blood clots[4] were heterotransplanted. Certain conclusions were at that time reached as to the action of hetero-toxins on the transplanted tissues and the mode of reaction of the host against the strange cells. There were, however, certain points, such as the behavior of the lymphocytes in heterotransplantations, which needed further analysis in order to arrive at a definite understanding of the factors active under these conditions. For this purpose we decided to extend these investigations to a tissue which, in our previous studies on homoiotransplantation, we found more resistant to injurious influences, namely, cartilage. Such a resistant tissue, we believed, might be especially suitable for the analysis of the reaction occurring in heterotransplantation. With the cartilage was transplanted the surrounding fat tissue. In conclusion we shall correlate the principal facts brought out in our various studies of heterotransplantation and thus arrive at a more complete analysis of the agencies active under these circumstances.

Five series of heterotransplantations of xiphoid cartilage were made. In the first and second one we transplanted rabbit cartilage to subcutaneous, ventrally situated pockets in the guinea-pig; in the third series we again used rabbit cartilage, but transferred it to subcutaneous, dorsal pockets in the rat, except in a few cases when we used ventral pockets. In the fourth series guinea-pig xiphoid cartilage was transplanted to dorsal pockets in the rat, and in the fifth series the reverse transplantations were carried out, that is, from the rat to ventral, subcutaneous pockets in the guinea-pig.

We shall discuss (A) the effect of heterotransplantation on the

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life of the transplanted tissue and on its regenerative power; (B) the reaction of the host connective tissue against the transplant; and (C) the behavior of the polymorphonuclear leucocytes and lymphocytes towards the transplanted tissue.

### A. The Duration of Life and the Regenerative Power of the Transplanted Tissue

We have to consider in this connection the cartilage, the perichondrium and the surrounding areolar and fat tissue. In Series I and II, which we shall discuss together, rabbit cartilage was transplanted to guinea-pig. The effects of the heterotransplantation were followed in a continuous order from the eighth to the twenty-fourth day after the operations. Throughout there is some cartilage and perichondrium preserved up to the last period at which we examined the pieces. The amount of preserved tissue varies in different pieces. On the whole, in those parts in which the tissue shows signs of degeneration, either slight or more marked chromatolysis of nuclei or shrinking of cells and nuclei are observed, but in various other places cells and nuclei are apparently perfectly preserved up to the last. Often the more centrally situated parts suffer most, but not infrequently the peripheral cells are more injured than the neighboring ones. In certain specimens we had the impression that the interstitial cartilage substance takes up fluid from the surrounding tissue and shows signs of swelling, a condition which would be analogous to the swelling of corneal tissue, observed under certain circumstances. This effect however needs further investigation. As to the fat tissue, it soon becomes to a large extent replaced by host connective tissue and infiltrated by lymphocytes and polymorphonuclear leucocytes; but some fat tissue may possibly remain preserved. In some cases there exists a correspondence between the necrosis of cartilage and surrounding fat tissue. While thus at the end of the period of observation chosen by us the heterotransplanted cartilage is at least in places preserved, there is nowhere any attempt at regeneration noticeable on the part of the perichondrium, and in this respect the heterotransplant differs definitely from the homoiotransplant, where regenerative processes are a general occurrence. We must therefore assume that the hetero-toxins cause invariably an injury of the transplant, even in places where the structure of the tissue is apparently normal.

In Series III in which we transplanted rabbit cartilage to the rat, conditions are on the whole similar to those found in Series I and II. Cartilage and fat tissue are partly well preserved, partly necrotic. Even as late as twenty and twenty-four days after operation, there are usually parts of the transplanted tissue apparently alive. On the whole, the preservation of cartilage, as well as fat tissue, is perhaps a little better in the rat than in the guinea-pig. As far as the fat tissue is concerned this may perhaps be due to the fact that in the rat, as we shall see later, the host tissue usually invades the transplant less actively than in the guinea-pig. It may also be that the place of transplantation has a slight influence on the fate of the transplant. In the rat we generally used dorsal and in the guinea-pig ventral pockets. But in several cases we made use of ventral pockets also in the rat without much difference in the results, except perhaps in one instance. However, in the rat there is found just as complete an absence of regeneration as in the guinea-pig. We observed here, as well as in the rabbit cartilage transplanted into the guinea-pig, that when the cells disappear in the hyaline cartilage substance, they leave there in some cases a fine system of lines indicating the former outlines of the cells, so that almost a honeycomb appearance is produced. In a specimen obtained twenty-four days after transplantation, we noticed a softening and solution of the intercellular cartilage substance with the preservation of the cartilage cells. The latter are thus found lying in the periphery of the preserved cartilage, joined to each other by long fine processes. Thus the character of a structure somewhat resembling myxoid tissue was obtained.

In Series IV in which guinea-pig cartilage was transplanted to rats, specimens were examined from the first day to the twenty-eighth day. Again we obtained the same results, but it seems here that after the completion of the first week following the operation, the necroses of cartilage and fat tissue increase gradually and are especially marked from the eighteenth day on. However, as late as the twenty-eighth day some tissue is apparently still preserved. On the whole, the necrosis is more extensive in heterotransplantation than in homoiotransplantation; but there are always variable factors of a more or less accidental character which influence the amount of necrotic material found.

In Series V (transplantation of rat cartilage to guinea-pig), the transplant is relatively well preserved in the first four days; but

some cartilage is necrotic, especially in the center of the tissue, in case a thick piece of cartilage has been used. As in the preceding series, the amount of living transplant gradually decreases, but some preserved cartilage may be found up to the last day of examination.

If we compare the effect of heterotransplantation on the life and proliferation of various tissues we come to the following conclusion: While skin, thyroid and kidney tissue become usually entirely necrotic after heterotransplantation sometime between the seventh and fourteenth days after the operation, cartilage remains alive much longer; four weeks was the latest time at which we examined heterotransplanted cartilage tissue and found still some parts at least structurally preserved. Cartilage, being generally more resistant than the other tissues used previously, is much less readily destroyed under the influence of hetero-toxins; however, it would probably only be a question of time until the whole cartilage would be replaced by host tissue. On the whole, the amount of necrosis in cartilage seems to be greater after hetero- than after homoiotransplantation; and it increases gradually with the time elapsing after the operation. There is active, evidently, a hetero-toxin which has a direct injurious effect on the heterotransplant.

While epithelial tissues are more sensitive to hetero-toxins than cartilage, the former show a certain number of mitoses as the result of stimulation through transplantation, which are noticeable usually until a few days before death of the piece; no mitoses appear under these conditions in cartilage or perichondrium, apparently in accordance with the fact that cartilage and perichondrium have normally no marked tendency to proliferation by mitosis. However, the proliferation which we notice in the perichondrium after auto- and homoiotransplantation and which tends especially to replace necrotic cartilage, is lacking after heterotransplantation as the result of the injurious action of the hetero-toxins.

### B. The Behavior of the Host Connective Tissue toward the Transplant

In homoiotransplantation of thyroid and various other organs and tissues, the connective tissue of the host actively invades the transplant and aids in its destruction. In heterotransplantation of kidney and thyroid, the connective tissue also surrounds the epithelial structures, forms hyaline tissue around them and through compres-

sion helps to destroy them. It may furthermore produce a capsule around the transplant as such. But on the whole the direct injury of the latter is so pronounced and the tissue is thus damaged to such an extent that the subsequent activity of the connective tissue plays only a relatively unimportant rôle in the process of destruction.

In heterotransplantation of cartilage the connective tissue of the host shows the following changes.

In Series I and II, rabbit cartilage was transplanted to the guinea-pig. Here the connective tissue is very active; from eight days on till the end, it invades fat tissue and replaces it gradually; soon it reaches the area surrounding the cartilage and forms a capsule about the latter. In various places, connective tissue cells and lymphocytes penetrate also into the cartilage, open the capsules of cartilage cells and fill them. At a period of twenty and twenty-four days following the transplantation, the connective tissue has invaded practically the whole transplant and replaced it, only a small part of the cartilage being left. Necrotic cartilage and probably also preserved cartilage have in this way been supplanted. At first, proliferation of the connective tissue begins at a distance from the cartilage; gradually it replaces more and more all of the tissue around the cartilage, presses on the latter and finally invades it. It is accompanied in its activities by lymphocytes and also by polymorphonuclear leucocytes. At early periods we may also see blood vessels extending through the transplant up to the cartilage.

In Series III, in which rabbit cartilage was transplanted into the rat, the activity of the connective tissue is on the whole less pronounced. The growth takes place mainly at a certain distance from the cartilage, but the connective tissue invades also fat tissue and in places supplants it. It penetrates the cartilage as well, especially where the latter is necrotic and where much hyaline intercellular substance has formed. In earlier periods especially (eight days after transplantation), blood vessels, with the connective tissue, almost reach the perichondrium. Conditions in the rat are then similar to those found in the guinea-pig, but on the whole the connective tissue activity is here somewhat less marked, except in a specimen examined fifteen days after transplantation into a ventral pocket, where the connective tissue activity resembles that observed in the guinea-pig.

In Series IV (guinea-pig cartilage into rat), the connective tissue

activity is distinct as early as three days after transplantation. At first, connective tissue with capillaries grows in the direction toward the cartilage; at later periods than the first week the blood vessels are not prominent. The connective tissue replaces gradually a great part of the fat tissue and penetrates also into the cartilage; but whether it penetrates preserved, as well as dead, cartilage is uncertain. As in homoiotransplantation, the cells move into the cartilage in the direction of the fibrils. The intensity of this connective tissue growth varies in different cases; in certain transplants only fibrous septa and nodules in the fat tissue are produced; in other cases the connective tissue replaces smaller or larger parts of the fat tissue.

In Series V (rat cartilage into guinea-pig), the connective tissue proliferation is noticeable two days after transplantation. The growth begins at first in the periphery of the transplant, but as early as ten days after the operation great parts of the fat tissue are replaced by fibrous tissue. Connective tissue begins also to invade the dead and probably also the preserved cartilage, but in various cases certain parts of the transplanted tissue may not yet be replaced by connective tissue as late as twenty-five days after the operation.

### C. The Behavior of Polymorphonuclear Leucocytes and Lymphocytes toward the Transplant

Series I and II, rabbit cartilage into guinea-pig. Throughout the time of observation, from five to twenty days after operation, polymorphonuclear leucocytes are present, occurring first in the transplanted fat tissue, where they collect in large numbers, more particularly in places which are necrotic. Soon they begin to penetrate also a little way into the cartilage and are seen here also mainly in necrotic areas. They are especially successful in the invasion of cellular cartilage, but find it more difficult to advance into the hyaline cartilage substance. Cartilage cells may be in part digested by them. They are also seen in the fibrous capsule around the transplant and in the fibrous tissue around the cartilage. In different transplants and at various points of the same transplant, the number of leucocytes varies considerably. They may be rare in one place and in another may produce abscess-like formations. In the rat, these cells are less numerous than in the guinea-pig in which they remain active throughout the whole period of observation. Lymphocytes were likewise visible as long as observations were made, from eight to

twenty-four days after transplantation. They appear first in the periphery of the transplant, but soon move through the fat tissue, surround the cartilage and often penetrate the perichondrium and peripheral cartilage. As early as eight days after transplantation they may be found around the cartilage. Lymphocytes, together with some polymorphonuclear leucocytes and connective tissue cells, may fill the spaces which are surrounded by the hyaline capsules. In general, lymphocytes accompany the connective tissue and collect in large numbers in areas of fibrous thickening around the cartilage and in the fat tissue. We do not find that they move freely toward and into the heterotransplant in the manner in which they may invade homoio- or syngenesiotransplants. As a rule the infiltration becomes very dense in the course of the second week after transplantation, but again we note variations in the density of infiltration in the individual transplants and at different points of the same transplant. Occasionally giant and epithelioid cells are observed in the fat tissue; but usually they are not a very prominent feature in the heterotransplant.

Series III, rabbit cartilage into rat. The number of polymorphonuclear leucocytes is much less in the rat than in the guinea-pig. From the tenth day on, these cells may be very rare or lacking altogether. They are found especially in necrotic tissue. We observed them around fat needles which had formed in the transplanted fat tissue. They may also be mixed with the collections of lymphocytes. The infiltration of lymphocytes follows, on the whole, the same rules in the rat as in the guinea-pig, but in general it is less marked in the rat. These cells appear first in larger numbers in the peripheral parts of the transplant, but they soon reach the cartilage and may penetrate the peripheral cartilage, especially in places where it is necrotic. In the third week the infiltration may become dense, particularly at a distance from the cartilage, but quite decidedly also around the cartilage. As usual, lymphocytes accompany the connective tissue and in the latter often thick infiltrations may form. Some large mononuclear cells are occasionally mixed with the lymphocytes. At times giant cells are seen in the remnants of transplanted fat tissue.

Series IV, guinea-pig cartilage to rat. During the first three days polymorphonuclear leucocytes are found in the fat tissue and around the cartilage, either diffusely distributed or localized; sometimes

they occur in dense masses, especially where fat is necrotic. Some of these cells may enter perichondrium or cartilage; in the latter they degenerate, owing to the unfavorable conditions. In necrotic muscle tissue there are many polymorphonuclear leucocytes. Four days after the operation they begin to decrease in number and then to disappear; at five days some are seen degenerating in the cartilage. In the fat tissue a certain number of scattered cells of this kind are observed. In the following period up to the tenth day, and probably also later, there are a few polymorphonuclear leucocytes in the connective tissue around cartilage, but they are less numerous than in the guinea-pig. On the whole there are more of these cells in heterotransplantation than in homoiotransplantation. As to the lymphocytes, some mononuclear cells appear in the fat tissue during the first three days after tranpslantation. After three days a few lymphocytes can be seen in this tissue and in necrotic cartilage; but they appear in masses only at four days at the periphery of the transplant in the proliferating connective tissue. Also in the fat tissue and in the necrotic connective tissue some lymphocytes are visible. From this time on, many of these cells appear in the growing connective tissue; but there are, besides, numerous scattered lymphocytes in the areolar tissue and in the fat tissue. After seven days there may be a moderate or a considerable lymphocytic infiltration around the cartilage and in the fat tissue. Lymphocytes penetrate also a little into necrotic cartilage. On the whole the lymphocytic infiltration is densest at a distance from cartilage, in the proliferating connective tissue. There is a similar distribution of lymphocytes at nine days, although these cells occur also in the tissue somewhat nearer the cartilage; directly around cartilage there are fewer to be found than at a distance from the cartilage, in the growing connective tissue. In the fat tissue, lymphocytes are arranged especially around vessels and in the fibrous tissue traversing the fat tissue. At ten and eleven days there may be distinct lymphocytic infiltration in the connective tissue around cartilage. Dense lymphocytic infiltration is usually seen in the fibrous tissue; otherwise in the fat tissue and in the perichondrium only scattered lymphocytes are observed. Some lymphocytes may even invade living cartilage. At fourteen days we found the cartilage surrounded by a mantle of these cells several times thicker than the width of the cartilage. They penetrate also into the perichondrium and even more deeply into

the cartilage where it is necrotic. However, the density of the lymphocytic infiltration varies in different pieces. From now on until the end of the fourth week the conditions are similar; there may be moderate or dense infiltration around the cartilage and the lymphocytes may invade the peripheral cartilage in the direction of the fibrils. After twenty-eight days lymphocytes and connective tissue penetrate into the necrotic cartilage. There may be a dense lymphocytic infiltration around the cartilage, even in places where there is no marked connective tissue growth. On the whole this infiltration is more pronounced in heterotransplantation than in homoiotransplantation; but in general, in the former it accompanies the new formation of connective tissue, although at later periods there may be marked lymphocytic infiltration more or less independent of fibrous tissue proliferation.

Series V, rat cartilage to guinea-pig. Polymorphonuclear leucocytes are found as early as twenty hours after transplantation; during the first three days they are seen in the fat tissue, especially where it is necrotic, and around the cartilage. They penetrate into the dead cartilage, but also into living tissue between living perichondrium and cartilage; we find them situated particularly around blood vessels, but may see rather dense collections in various places. They are more numerous in these heterotransplants than in homoiotransplants. In the following period they diminish in number less markedly in the guinea-pig than in the rat. Thus at ten days, polymorphonuclear leucocytes migrate through necrotic fat tissue towards perichondrium and cartilage. They may be arranged diffusely or in localized collections. In the fat tissue and around cartilage some of them may disintegrate. The same condition is found in later periods up to the twenty-fifth day, but more and more lymphocytes begin to prevail, especially where a new formation of connective tissue has taken place. There are, however, polymorphonuclear leucocytes mixed usually with the lymphocytes and particularly in areas of fat tissue, the former cells may be seen almost exclusively; these may themselves become necrotic. The lymphocytes appear somewhat later than the leucocytes. Two days after transplantation we find a collection of lymphocytes and some larger mononuclear cells in the periphery of the transplant. Somewhat later they occur in considerable number, at a distance from the cartilage where the connective tissue is proliferating, and from here they penetrate into the

fat tissue. Thus in the second week there are found more or less dense collections of lymphocytes in the growing connective tissue. These cells now penetrate, on the one hand, somewhat outwardly into the adjoining muscle tissue of the host and, on the other hand, in the direction of and a little distance into the dead or living cartilage. There may develop considerable accumulations of lymphocytes in the fat tissue and also around the cartilage, especially where connective tissue is growing. At fifteen days we see in the fibrous tissue around the cartilage lymph vessels filled with lymphocytes and a marked lymphocytic infiltration extends to the cartilage. From the eighteenth to the twenty-fifth day we may find a similar dense or moderate infiltration in the connective tissue around the cartilage and in the fat tissue and at this time lymphocytes are therefore often very prominent.

### Comparison between Homoiotransplantation, Transplantation into Different Varieties, and Heterotransplantation in the Rat

It is of interest to compare the reactions, in these three types of transplantation, of a relatively resistant tissue like cartilage. At twenty or twenty-one days after transplantation, there is usually, in ordinary homoiotransplantation, much connective tissue growth and this tissue generally replaces considerable parts of the fat and areolar tissue. The lymphocytic reaction is noticeable; but both connective tissue and lymphocytic reactions vary in intensity in different pieces. There may be some necrosis in the transplant, especially in the central parts, but the latter may show degenerative changes even in the normal, not transplanted xiphoid cartilage; areas of solution also occur. In other places as well, areas of cartilage may be necrotic. Bone marrow is replaced by fibrillar connective tissue. Regenerative new formation of cartilage on the part of the perichondrium is, in many cases, quite marked around necrotic areas in the transplanted cartilage. If we compare with the results of these typical homoiotransplants, those of the transplantation of cartilage into rats with different color pattern, we find, in general, that the amount of necrosis of transplanted tissue is greater in the latter case. Perichondrial new formation of cartilage is here very rudimentary or absent. Transplanted bone is necrotic, while in homoiotransplantation it may be partly preserved. The new formation of connective tissue

and replacement of fat tissue by fibrous tissue are more pronounced than in homoiotransplantation and the lymphocytic reaction is likewise more marked.

In heterotransplantation, the direct injury of the transplanted cartilage is usually greater than in either of the other two kinds of transplantation. There are as a rule more degenerative changes and areas of necrosis in the cartilage and perichondrium. Not rarely we find that especially the peripheral parts of the transplant are injured to a greater extent than the central parts. While in homoiotransplanted cartilage there may be areas of solution in the cartilage, these are usually lacking in the heterotransplants, although we found in the periphery of one of the latter transplants hyaline cartilage substance dissolved and the preserved cartilage cells forming a myxoid-like tissue. Perichondrial new formation of cartilage as well as the formation of nuclear chains, which are observed in homoiotransplanted striated muscle, are entirely lacking in heterotransplantation. On the other hand, here the formation of connective and especially of fibrous tissue is most pronounced, and the fat tissue in particular is in the course of time more and more replaced by fibrous tissue produced by the host. Lymphocytic infiltration is also very prominent in heterotransplantation. One characteristic feature here is that this infiltration is usually associated with connective tissue proliferation; this may be due to the fact that the latter process is so very marked in heterotransplantation. Furthermore connective tissue and lymphocytes usually penetrate together into injured parts of the cartilage; however, lymphocytic infiltration may occasionally be pronounced in places, when connective tissue new formation is apparently not very prominent. On the whole, it may be said that lymphocytic infiltration is more intense in hetero- than in homoiotransplantation or in transplantation into different varieties. In the latter two types, polymorphonuclear leucocytes are lacking at this period, while they are usually found in the first type, although in smaller number in the rat than in the guinea-pig.

Between five and eighteen days we find in principle the same differences between homoio- and heterotransplantation. The lymphocytic infiltration in the latter case is, in the earlier periods, especially marked at some distance from the cartilage and in the more peripheral parts of the transplants. At seven days the transplants into different varieties are already in some respects distinct

from homoio- as well as from heterotransplants. As compared with homoiotransplants, we find in the case of transplantation into strains with different color pattern (variety transplants) a greater amount of necrosis and an absence of perichondrial regeneration; there is also a beginning connective tissue activity and a moderate lymphocytic infiltration, the latter being more marked at this period than in the average case of homoiotransplantation. In heterotransplants on the other hand, the necrosis of the transplanted tissue, as well as the connective tissue proliferation and probably also the lymphocytic infiltration, is still more marked. There are also more polymorphonuclear leucocytes present in the heterotransplant, the greatest intensity of invasion by these cells being especially observed in the first few days; some leucocytes, however, may be seen even in the homoiotransplant in the first three days after operation.

We may then conclude that in (1) homoiotransplantation, (2) transplantation to different varieties and (3) heterotransplantation there is a gradation of reactions of the host and a corresponding gradation of effects on the transplant which become the more severe the more distant the genetic relation between host and transplant.

## The Reactions of the Host against Heterotransplanted Tissues in General

We have studied the effect of heterotransplantation on various tissues, namely skin, thyroid, kidney and cartilage. In all these cases we were able to compare heterotransplantation with auto- and homoiotransplantation. Furthermore, we carried out experiments on homoiotransplantation of blood clots and we compared it, in some additional experiments, with heterotransplantation of blood clots. Thus we can, on the basis of these observations, arrive at some more general conclusions concerning (1) the effect of hetero-toxins on tissues and (2) the reactions of the host cells against heterotransplants. As far as heterotransplantation of blood clots is concerned, the number of our experiments is as yet relatively small and the conclusions must therefore at present be considered as provisional.

1. The effect of hetero-toxins on tissues. If we transplant living tissue into a different species, there is noticeable a direct injurious effect of the body fluids on the transplant. Under these conditions, some constituents of the body fluids act as hetero-toxins. This is

true for all tissues so far investigated by us and it may even hold in the case of blood clot, where hemolysis seems to be more marked after hetero- than after homoiotransplantation. However, the power of resistance of various tissues varies. Skin, thyroid and kidney die between the seventh and fourteenth day after heterotransplantation; bone marrow and striated muscle tissue die rapidly after heterotransplantation; fat tissue also perishes, but in this case it is difficult to determine the exact time of complete necrosis. Cartilage, on the other hand, may remain preserved in part for four weeks or perhaps even somewhat longer; at least there are parts of this tissue which behave toward ordinary stains like normal tissue. Even in the case of cartilage, however, the amount of necrosis is greater after heterotransplantation than after homoiotransplantation or after transplantation into different varieties. It is known that certain transplantable tumors can remain alive and grow for an even longer period than cartilage, if the transplantation has been made into an animal of a different but not very distant species. However, after heterotransplantation, even those parts of tissues which are morphologically preserved are injured more intensely than after homoiotransplantation. This may be concluded from the fact that regenerative phenomena are diminished or lacking altogether after heterotransplantation. Tissues, such as skin, thyroid and certain tubules of the kidney, which naturally are more susceptible to growth stimuli, or which respond more readily to growth stimuli with mitoses, may still show mitoses after heterotransplantation, although these are less numerous than under more favorable conditions. Usually they cease a few days before the tissue becomes entirely necrotic; however, some mitoses may still occur near the time of death. In the case of perichondrium or peripheral cartilage, on the other hand, in which under favorable conditions regeneration takes place in response to the stimulation exerted by necrotic cartilage tissue and in which mitoses are rare and the tendency to cell proliferation is apparently less pronounced than in the case of certain other tissues, no trace of regeneration has been observed by us after heterotransplantation. Also, in heterotransplanted striated muscle, the formation of nuclear chains, which may be seen after auto- and homoiotransplantation, is completely lacking. Homoio-toxins, on the other hand, affect directly only very sensitive tissues, like the myxoid connective tissue of the uterus, unstriated muscle tissue and

probably bone marrow, while some other tissues are rather resistant to homoio-toxins. But all these tissues are severely affected by hetero-toxins. We may then conclude that hetero-toxins produce much more intensely a direct injurious effect on transplanted tissues than do homoio- or syngenesio-toxins. This effect is noticeable in a diminution in regenerative processes as well as in a relatively rapid destruction of tissue; however, certain differences exist in this respect between the various tissues and the naturally more resistant tissues have also a greater power of resistance to the action of hetero-toxins.

2. If we now consider the reactions of the host tissues against the transplant, we find again essential accordance in the different kinds of heterotransplantations, but certain variations occur due to conditions of secondary importance. In all cases the connective tissue proliferates and has a tendency to form fibrous tissue around and between the structures of the transplant, pressing upon the latter and thus injuring it. It invades and largely replaces transplanted fat tissue. Cartilage also is invaded, especially necrotic areas; but apparently certain parts at least partially preserved are invaded as well. In the case of cartilage, furthermore, we found that fibrous tissue forms in larger quantity around the transplants than around transplants of thyroid or kidney. This is probably due to the fact that cartilage is less rapidly destroyed than the two latter tissues and it can thus exert an effect upon the host which extends over a longer period of time; however, we saw the same reaction also during the second week in a few cases of thyroid and kidney transplantations.

A further reaction of host to transplant consists in the collection of large masses of lymphocytes in the connective tissue at some distance from the transplanted piece. This as well as the connective tissue proliferation we notice especially when cartilage and fat are used, although as stated, we find it indicated also in the case of the other tissues. Having infiltrated densely the area around the transplant, especially during the second week, the lymphocytes then invade it together with the connective tissue. Cartilage, it will be remembered, resists to some extent the action of both of these agencies, at least for some time, though very considerable masses of lymphocytes may collect around the transplant. In the case of skin, thyroid and kidney heterotransplants, the invasion by lymphocytes is much less pronounced than when homoiotransplants are made;

but even here lymphocytes may during the second week begin to collect in the periphery in large masses. The reasons for a less active lymphocyte invasion of such hetero-tissues as the tubules of the kidney and the acini of the thyroid are: (1) the earlier death of heterotransplants as compared with homoiotransplants; during the second week the former have to a large extent been destroyed; (2) the diminished metabolism generally of heterotransplants; and (3) their imperfect vascularization, lymphocyte infiltration taking place largely by way of the lymph vessels invading the transplant.

As far as we can judge, the heterotransplanted blood clot behaves in a manner similar to the other heterotransplants, especially cartilage and fat: it is surrounded and gradually replaced by large masses of fibrous tissue and lymphocytes. It seems that relatively inert foreign bodies, such as agar and serum coagulated through the action of heat, become organized without calling forth a similar, very marked proliferation of connective tissue and a massing together of lymphocytes. However, in the case of connective tissue, its activity as far as the heterotransplant is concerned consists in all probability largely in a migration of fibroblasts toward and into the transplant rather than in their mitotic proliferation.

We must, therefore, conclude that specific toxic substances, the hetero-toxins, are given off by these transplants which cause a reaction on the part of the connective tissue and lymphocytes of the host. Furthermore, if we consider that hetero-blood clots and, apparently, also the dying or necrotic tissue may call forth these reactions, it becomes probable that the hetero-toxins do not depend for their activation on the characteristic metabolism of living tissue, as it seems homoio-toxins do. This conclusion would be in harmony with the fact that while immunization against homoio-tumors can be made only with living tissue, we can accomplish such an immunization against heterotransplants also with lifeless material. There is an additional difference between homoio- and hetero-toxins: the latter attract the polymorphonuclear leucocytes much more markedly than the former. After homoiotransplantation we notice only a few polymorphonuclear leucocytes in the first few days after transplantation, while after heterotransplantation they are usually more numerous and they persist much longer. To some extent these reactions of the host against heterotransplants are also influenced by the specimens into which they are transplanted. Thus

in the guinea-pig, the activity on the part of the leucocytes and probably also on the part of the lymphocytes is more pronounced than in the rat. Reciprocal heterotransplantations, reversal of host and donor, do not necessarily give the same results, as we found in our earlier heterotransplantations. The reaction depends upon the activity of the host, which is not affected equally by all kinds of differences between host and transplant, but by differences of a special kind.

## Summary

1. The direct injurious action of the body fluids of the host on cartilage and fat tissue which have been transplanted into a strange species is greater than the corresponding effect following homoiotransplantation. This direct injury has been observed by us in various tissues which we examined after heterotransplantation. However, cartilage differs from other tissues through its greater resistance to injuries in general and to the action of hetero-toxins in particular. Heterotransplanted cartilage may remain in part preserved for at least four weeks; but even the apparently intact parts, as far as structure is concerned, are injured inasmuch as no regenerative growth of perichondrium is observed.

2. The reactions of the host against the heterotransplanted cartilage and fat tissue are pronounced. A very marked invasion of the fat tissue by connective tissue takes place; the latter then becomes fibrous and replaces the fat tissue to a large extent. The cartilage is enveloped by fibrous tissue and partly invaded, especially in places where it is injured. Large masses of lymphocytes gradually collect around the cartilage, the infiltration beginning at a distance from the cartilage but gradually affecting the greater part of the transplant. This infiltration is generally associated with the connective tissue invasion of the piece, but occasionally it may occur in certain places to some extent independently of the connective tissue. After heterotransplantation, polymorphonuclear leucocytes collect in large numbers in and around the transplant and in the connective tissue, invading the transplant in larger numbers than after homoiotransplantation. These cells also persist much longer around heterotransplants than around homoiotransplants, where they are usually found only during the first three days following transplantation.

3. Some quantitative variations exist in different species in the

intensity of the reaction against heterotransplants; but in principle the reaction is the same in all species examined.

4. Some differences also exist in the behavior of the various tissues after heterotransplantation; but these are due mainly to differences in the resistance of the tissues, their tendency to mitotic proliferation and the duration of their survival. In principle the reactions are the same in the case of all heterotransplanted tissues tested so far.

5. There are some indications that while in the case of homoiotransplants only actively metabolizing tissue elicits the reaction on the part of the host tissue, in the case of heterotransplants dying or dead tissue also may call forth a reaction.

6. In comparing the results of transplantation of tissues into different, not related, individuals, into different varieties and into different species we find a gradation in the directly injurious effects on the tissues as far as their preservation and power of regeneration are concerned, as well as a gradation in the intensity and character of the reaction of the host against the transplants. Connective tissue and polymorphonuclear leucocytes react most strongly against heterotransplants, while blood and lymph vessels grow least into heterotransplants. Lymphocytes surround and invade transplants into different varieties more actively than homoiotransplants; they are very active around heterotransplants, provided that the destruction of the latter does not proceed with such rapidity that the attracting substances given off by the heterotransplants are correspondingly diminished in quantity.

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# A CASE OF CARCINOMA OF THE COLON ASSOCIATED WITH SCHISTOSOMIASIS (BILHARZIOSIS) IN A YOUNG WOMAN *

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It is well known, especially from observations in Egypt by Ferguson,[1] that carcinoma of the bladder frequently supervenes upon endemic hematuria, a disease caused by the invasion of the bladder by the eggs of the digenetic trematode, *Schistosomum haematobium*. In view of this, there is a general impression that schistosomiasis (bilharziosis), may be an inciting factor of carcinoma. Schistosomiasis has also been etiologically connected with certain cases of primary carcinoma of the liver by Yamagiwa [2] (*Sch. Japonicum*), by Mouchet and Fronville,[3] and especially by Pirie.[4] Intestinal schistosomiasis, although it may give rise to extensive circumscribed thickenings of the mucosa of the colon in the form of polyps or papillomas (Sinderson and Mills,[5] Dolbey and Fahmy,[6] Martinez[7]), shows no special tendency to be followed by carcinoma, as far as can be seen from the literature. Therefore, the case of associated schistosomiasis and carcinoma to be reported below, having occurred in an individual who was far below the usual cancer age, seems sufficiently striking to be of interest in this connection.

## CASE REPORT

Maria M. (16398), a Porto Rican native, 18 years old, single and a cigarette maker by occupation, was admitted to the Presbyterian Hospital, San Juan, Porto Rico, Jan. 4, 1926, complaining chiefly of abdominal pain and distension. She stated that these symptoms first appeared two months ago and that they have gradually become very severe. During the last month she has also vomited almost every day and she has had no movement of the bowels for fifteen days. The family history of the patient is irrelevant. From her past history it appears that she had had measles and smallpox in child-

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hood; she also claimed to have had hookworm disease two years ago.

On examination the patient appeared acutely ill, her temperature was 99.2 F and the pulse rate 120 per minute. Nothing remarkable was found anywhere except in the abdomen. The latter was found considerably distended, somewhat rigid and tender throughout. Suspecting intestinal obstruction, the patient was operated upon immediately (January 4). On opening the abdomen (Dr. Galbreath), much fluid escaped and a mass was found involving the splenic flexure of the colon, apparently producing complete obstruction. A loop of ileum was adherent to the mass. The large intestine as well as the ileum was considerably distended down to the mass. Below the latter the colon was collapsed. The ileum was also discolored and its serosa as well as that over the mesentery was covered with plastic exudate. Because of the poor condition of the patient no attempt was made to resect the colon and relief from the obstruction was attempted by means of colostomy.

The patient returned from the operating room in a condition of profound shock and died at 6 P.M. A necropsy was not obtained, but the wound was reopened postmortem and the entire mass was resected from the colon.

*Pathologic Findings.* Grossly, the resected mass represents a funnel-shaped portion of the colon, measuring 12 cm. in length. About two-thirds of its length is occupied by a tumor mass which almost completely fills the lumen, allowing only a thin probe to pass with difficulty. In that region the wall is hard and retracted, showing on section almost complete replacement of the wall by a grayish tumor mass irregularly studded with many gelatinous areas. The intestinal mucosa, both above and below the tumor mass, is reddened and thickened. Several lymph nodes of the mesocolon are enlarged and indurated — obviously carcinomatous.

Microscopically, the tumor shows the picture of a colloid (gelatinous) adenocarcinoma in its central portion where the intestinal wall is almost entirely substituted by tumor elements and massive accumulations of mucus. In the peripheral parts it appears more like a diffusely infiltrating carcinoma, consisting of clear spherical cells distended with mucus and of various sizes but mostly large. Their nuclei are distorted and pushed to the periphery (signet-ring cells). The stroma of the tumor in these regions is mostly poorly

developed, but in some places it is quite abundant, giving the character of a scirrhus. The lymph node metastases show everywhere the picture of a diffusely infiltrating carcinoma composed almost entirely of signet-ring cells with very little stroma. This type of tumor and its variations being well known, it is not necessary to go into further detail of its structure.

The unusual microscopic feature in this tumor is the presence of an abundant number of lateral spined ova which are readily identifiable as the ova of *Schistosoma mansoni*. These are also found in the metastatic foci of the lymph-nodes, but they are especially numerous in the peripheral parts of the tumor and in the adjacent parts of the colon. Here in addition to eggs there are also found adult worms in many places in the veins of the submucosa. In a few of the ova the spines appear at the pole as in *Schistosoma haematobium* but after careful observation it seems probable that such eggs are the result of artificial displacement of the spines during the process of hardening and embedding. This is apparent from the distorted shape and displacement of the embryo within the eggs. At any rate, on dissolving pieces of tissue with caustic soda solution, many typical lateral spined ova are recovered and no structures identifiable as ova contain processes which can be interpreted as polar spines. The eggs are in various stages of development, many of them containing full grown miracidia and showing various stages of disintegration. In the peripheral parts of the tumor where the different layers of the intestine are still well preserved, the ova are especially numerous in the mucosa and submucosa, and scarce in the muscular coats. In the more central parts of the tumor they are present mostly in those regions where the stroma is well developed. They are infrequent where the mucus is most abundant.

The reaction of the tissue toward the parasites is the same in all parts of the tumor as well as in the surrounding uninvolved parts of the intestine, and conforms to the tissue changes generally described for schistosomiasis (Dew,[8] Martinez,[7] and others). The most common reaction found is in the form of a well circumscribed nodule of about the size of a miliary tubercle consisting only of young fibroblasts, in the center of which is enclosed the more or less degenerated ovum. Very frequently the fibroblastic areas are surrounded by a varyingly thick wall of small round cells. Occasionally such a nodule contains more than one ovum (two to five), and not infre-

quently one or more foreign body giant cells can be found encroaching upon the ovum within the tubercle ("bilharzial tubercle").

In many places also, the fibroblastic reaction is missing and the eggs seem engulfed entirely in a mass of small round cells. In other places the reaction seems more acute and consists chiefly of an infiltration with eosinophilic polymorphonuclear leucocytes for a considerable distance around the ovum. Such reactions are most frequent about the eggs in and around the mucosa, while in the muscularis, eggs are often encountered which hardly show any cellular reaction around them, being enveloped only in a thin fibrous tissue capsule. Another reactive change which must be ascribed to the invading parasites is the enormous thickening of the mucosa in the parts of the intestine immediately adjoining the tumor. This is due to hypertrophy of the glandular crypts as well as to a dense infiltration of the stratum proprium with neutrophilic as well as eosinophilic leucocytes, lymphocytes and plasma cells.

The destructive changes in the ova are most prevalent in the thickest parts of the tumor. Here the fibrous tubercles frequently show only the chitinous shells of the eggs or their fragmentary remains. Total or partial calcification of the eggs is comparatively uncommon, and while the best preserved ova are to be found mostly in acutely inflamed areas, lytic processes in many embryos of these areas are quite apparent.

## Comment

The relation of the schistosomiasis to the carcinoma in this case is, of course, impossible to determine. From certain considerations it would appear that the two conditions might well be regarded as a coincidence. On the one hand, carcinoma in young individuals is probably of much more frequent occurrence than is usually assumed, as can be seen especially from a very recent study by Quensel.[9] Moreover, according to the statistics of the same author as well as those of others (Philipp,[10] Merkel,[11] and Staemmler [12]), the carcinoma of young individuals is most frequently an intestinal carcinoma. On the other hand, in localities where intestinal schistosomiasis occurs, carcinoma of the colon is, as mentioned above, not known to be especially common. Ferguson [1] who has pointed out the frequency of secondary carcinoma of the bladder to bilharzial lesions in Egypt, rather emphasizes the rarity of carcinoma of the

colon in the same country, although intestinal schistosomiasis is more liable to be accompanied by neoplastic changes. Nor does colon carcinoma appear to occur with especial frequency in Porto Rico where the index of intestinal schistosomiasis is, according to Martinez,[7] 2.16 per cent (Utuado) to 8.4 per cent (Mayaguex).

Yet the significance of metazoan animal parasites, especially helminths, as tumor-inciting agencies regardless of the nature of their action, cannot be doubted. This has been amply demonstrated experimentally (Fibiger,[13] Bullock and Curtis[14]). As to schistosomiasis, the notion that it is conducive to carcinoma in man seems well founded even judging only from the bladder carcinoma which follows endemic hematuria. In intestinal schistosomiasis, so-called precancerous lesions are common enough. Perhaps the failure of carcinoma to develop more frequently in the intestine than in the bladder is due to a more effective defensive power of the intestine against these particular parasites. Thus after considering the lessened carcinoma incidence for the age of the patient in our case together with the tumor-inciting properties of the parasites, the possibility that the latter were in some way responsible for the carcinoma, can hardly be disregarded.

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## DESCRIPTION OF PLATES

### PLATE 100

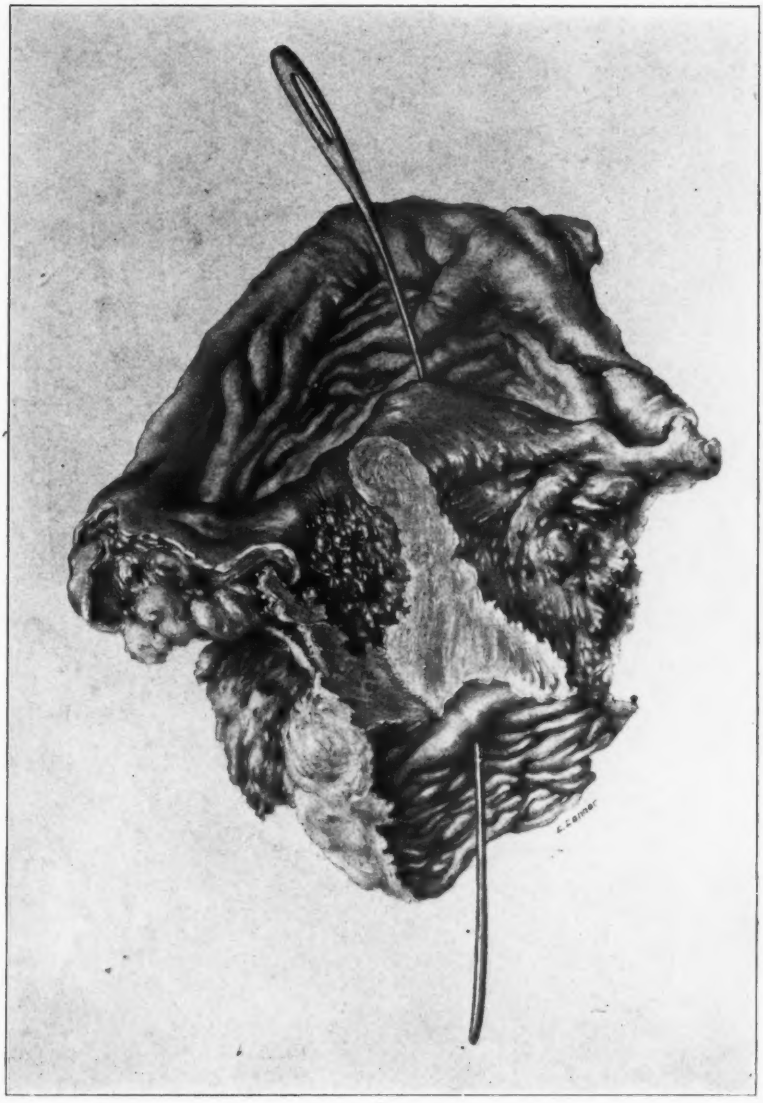
FIG. 1. Gross appearance of the resected colon, showing the tumor, with a probe in the stenosed lumen, and a tangential section of the wall.

### PLATE 101

FIG. 2. Microscopic appearance of the tumor in its central parts (adenocarcinoma).
FIG. 3. Cross-section of a vein containing a worm (male and female) in a scirrhous part of the tumor.

### PLATE 102

FIG. 4. Section of a submucosal vein containing three worms.
FIG. 5. Typical fibroid "tubercle" containing two ova and adjacent to it a nest of tumor cells surrounded by a wall of small cell infiltration.
FIG. 6. "Bilharzial tubercle" with a giant cell encroaching upon a lateral spined ovum.



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Roman and Burke Carcinoma Associated with Schistosomiasis

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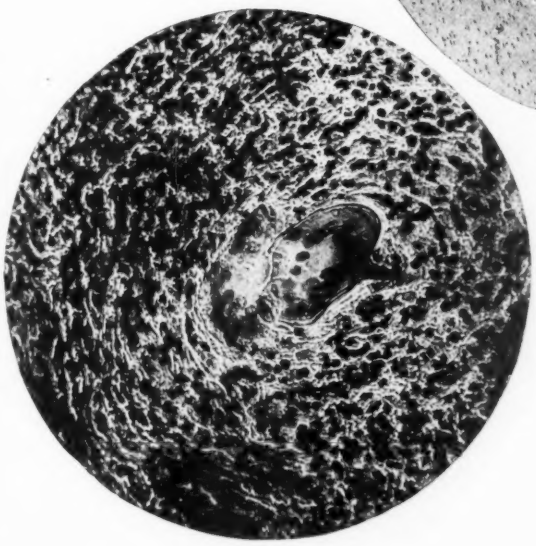

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Roman and Burke Carcinoma Associated with Schistosomiasis

# A STUDY OF THE TUMOR INCIDENCE IN THE LOWER ANIMALS *

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One of the greatest obstacles to a proper study of tumors in the lower animals is the lack of statistical data as to the total incidence in the various species of the different types of the disease. There is a great wealth of statistical evidence dealing with tumors in man but veterinary literature is woefully lacking in this regard. This is due largely to the fact that unlike the student of human medicine, the veterinary pathologist has no central agency of vital statistics to analyze and tabulate the results obtainable from death certificates. This information must come from animal clinics such as veterinary hospitals and institutions where accurate records are kept. The individual veterinary practitioner can contribute but little in this regard, although his carefully kept records for a number of years, if available, would have considerable value.

In reviewing the meager data on the frequency of tumors in the lower animals one is impressed with the fact that these formations are fairly common. The frequency of their occurrence is indicated by Kinsley's statement [1] that of 127 animals presented at the clinic of the Kansas City Veterinary College during one term, twelve were affected with neoplastic growths.

H. Martel, Chief of the Sanitary Veterinary Service, city of Paris, in an annual report [2] gives the results of statistical studies on the frequency of cancer among horses killed in abattoirs. Of 39,800 carcasses examined, mares constituted 2,000, geldings numbered 16,200, while 3,600 were stallions. Out of this number 184 were affected with cancer, distributed as follows: 86 in mares, 43 in geldings and 55 in stallions. The melanotic sarcomas were not counted since, Martel writes, "It is extremely rare to find white or gray horses entirely free from melanotic tumors." Most of the cases

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This is a condensed summary of a thesis presented to the Colorado Agricultural College in partial fulfillment of the requirements for a Master's degree.

were in subjects 15 years or older, while 118 horses of dark color were affected and 66 were found in whites and grays. Generalization was observed in 66 cases. As to locations affected, Martel found the 184 cases distributed as follows: kidney, 62; testicles, 50; mammae, 45; intestines, 9; bladder, 6; ovary, 2; lungs, 2; uterus, 1; sheath, 1; jaw, 1. The locations of five were not established. As to multiplicity, both testicles were involved in ten of the fifty cases, while out of the forty-five mammary tumors both glands were affected in six cases. The above figures are valuable but would be more so, if the various histologic types found were classified. From these figures it is evident that about one-half of 1 per cent showed malignant epithelial tumors and since cancer alone is mentioned, the total tumor incidence must have been much higher. The frequency of the disease in the kidneys, mammae and testicles is interesting for in these organs were located 157 out of the 184 cancers reported. Martel's figures also show a possible relationship between pigmentation and cancer since nearly two-thirds of the tumors occurred in the dark coated animals. This conclusion is perhaps not justified for no information is given as to the total number of dark and light horses respectively in the entire series studied (39,800).

Statistics from the Veterinary High Schools of Berlin, Dresden and Munich [3] show that 1.5 per cent of all horses, 4.5 per cent of all dogs, and nearly 20 per cent of all bovines presented for treatment were suffering from tumors.

Bemis [4] of Iowa reported that out of a clinic of 2,754 surgical cases, true tumors were observed in 27 per cent with about one-fifth of the cases malignant. Bemis' figures on incidence are considerably higher than others available.

In Dr. Kingman's clinic at the Colorado Agricultural College, in one series of 3,000 cases there were thirty-one cases in which true tumors were observed. This compares favorably with the German figures first quoted.

A surprisingly large number of fowls are victims of tumors and while reliable statistics dealing with this phase of tumor incidence are scarce, every poultry pathologist realizes that the percentage must be high; higher in fact than in mammals. In a recent article by Schneider [5] based on necropsies of all birds dying in a population of 11,000 individuals she reports the annual tumor rate for fowls between the ages of 6 and 18 months to be between 2 and 3 per cent.

Trotter [6] made an interesting report on malignant growths in the bovine from his study of 300 cattle suffering from malignant tumors. In the 300 individuals Trotter found 279 carcinomas and twenty-six sarcomas. (The difference in the total number of tumors in this instance was due to the fact that three of the animals had two primary growths while in one three primary tumors were found.) Only two of Trotter's cases were males (steers) while 298 were cows. Three cases were animals from 1 year to 3 years old while the rest, 297, were "aged." The locations of the tumors in Trotter's series were as follows: liver, 222; rumen, 25; thymus, 16; intestines, 10; lung, 8; ovaries, 5; bone, 1; skin, 1; eye, 4; vulva, 3; lymph glands, 3; kidney, 1; gall bladder, 1; uterus, 1; fascia, 1; salivary glands, 1; undetermined, 2.

The above statistics, while not in any sense exhaustive, should be sufficient to emphasize the fact that tumors of the lower animals are not uncommon but instead that they are rather frequently seen. The diagnosis of internal neoplasms in animals has not achieved the refinement practiced by the human physician and as a consequence the student of animal pathology is materially handicapped in having to depend largely upon an occasional necropsy for the chance discovery of internal tumors. Most animals dying on the farm are never examined postmortem and as a result comparatively few internal tumors are reported. In human medicine, on the other hand, internal neoplasms are usually diagnosed during life and an operation undertaken to rid the victim of the growth. To the number of tumors added to the human statistics from this source we have not a few added as the result of postmortem examinations to determine the cause of death in some obscure ailment. Then again, there is a greater interest on the part of the human physician and surgeon because of the attention neoplastic diseases command by their position in the annual mortality rates. Any disease that annually causes the death of between 90,000 and 100,000 of our population stimulates a certain earnestness on the part of those charged with the alleviation of human suffering and as a consequence we notice a coöperative effort toward the common goal that veterinarians usually fail to show.

While tumors of the lower animals do not occupy the same relative position of importance as tumors of man yet there is enough of practical worth to be learned to warrant a more sympathetic attitude on

the part of veterinarians in general toward this neglected field of pathology.

With the view of contributing something of value to the rather meager available information dealing with the histopathology, incidence, points of origin and location of the various groups of tumors as they occur in the lower animals, a series of studies was started some four years ago.

The work consisted of a thorough examination of all neoplastic material from the lower animals that could be obtained, the various specimens being studied grossly and microscopically, photomicrographs made and a diagnosis arrived at, using Mallory's nomenclature as far as possible. This report deals with a summary of the results up to this time.

In order to solicit the assistance of those most likely to encounter tumors in their daily routine, a form letter was addressed to thirty-five practicing veterinarians, most of whom were located in Colorado. Invitations to send in material were also sent to a half dozen or so of our graduates in the Government Meat Inspection Service and to a few veterinarians employed in clinical laboratories. In addition, the assistance of Drs. H. E. Kingman and James Farquharson of our college veterinary hospital was secured.

The response from the practitioners was somewhat disappointing. Ten or twelve have sent in one specimen, while four or five have supplied a goodly number. A large share of the material was obtained from packing house cases, although not a few were obtained through the kindness of other laboratories and our college veterinary hospital. Our own laboratory during the past two years has been a fruitful source, particularly of chicken tumors.

A summary of the source of the material received is as follows:

| | |
|---|---|
| From practicing veterinarians | 45 |
| From packing house sources | 38 |
| From the College Veterinary Hospital | 25 |
| Other laboratories | 33 |
| Our laboratory | 16 |
| Total specimens received | 157 |

This total included a number of specimens that were found not to be tumors when subjected to a microscopic examination and others that were not suited for study due to the lack of preservation and fixation. The total number of actual tumors studied was 132.

The 132 tumors studied were distributed among the various species as follows:

| Kind of tumor | Mule | Horse | Dog | Bovine | Sheep | Swine | Fowl | Mouse | Rabbit | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Fibroma | 3 | 5 | . | 5 | . | . | . | . | . | 13 |
| Fibrosarcoma | 3 | 3 | 1 | 2 | . | . | . | . | . | 9 |
| Hypernephroma | . | .. | . | 4 | . | . | . | . | . | 4 |
| Lipoma | . | 1 | 1 | .. | . | . | . | . | . | 2 |
| Leiomyoma | . | .. | . | 2 | . | . | 1 | . | . | 3 |
| Endothelioma | . | .. | 1 | 1 | . | . | 1 | 2 | . | 5 |
| Mesothelioma | . | 1 | . | 1 | . | . | . | . | . | 2 |
| Myxoma | . | .. | . | 1 | . | 1 | . | . | . | 2 |
| Myxofibroma | . | .. | 1 | .. | . | . | . | . | . | 1 |
| Melanoma | 1 | 2 | . | 4 | . | 2 | . | . | . | 9 |
| Melanosarcoma | . | 2 | 1 | .. | . | 1 | . | . | . | 4 |
| Lymphocytoma | . | .. | 1 | .. | . | . | 9 | . | . | 10 |
| Lymphosarcoma | . | .. | 4 | 2 | 2 | 2 | 2 | . | 1 | 13 |
| Lymphoma | . | .. | . | 1 | . | . | . | . | . | 1 |
| Embryonal Carcinoma | . | .. | 1 | .. | . | . | . | . | . | 1 |
| Carcinoma | . | 11 | 1 | 15 | . | . | 1 | . | . | 28 |
| Adamantinoma | . | 1 | . | .. | . | . | . | . | . | 1 |
| Adenocarcinoma | . | 1 | 1 | 2 | . | 9 | 3 | . | . | 16 |
| Adenoma | . | .. | 3 | .. | . | . | 2 | . | . | 5 |
| Papilloma | . | .. | 1 | 1 | . | . | . | . | . | 2 |
| Cholesteatoma | . | 1 | . | .. | . | . | . | . | . | 1 |
| Totals | 7 | 28 | 17 | 41 | 2 | 15 | 19 | 2 | 1 | 132 |

The epithelial tumors occurred as follows:

| Kind of tumor | Mule | Horse | Dog | Bovine | Sheep | Swine | Fowl | Mouse | Rabbit | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Carcinoma | . | 11 | 1 | 15 | . | . | 1 | . | . | 28 |
| Adenocarcinoma | . | 1 | 1 | 2 | . | 9 | 3 | . | . | 16 |
| Adamantinoma | . | 1 | . | .. | . | . | . | . | . | 1 |
| Adenoma | . | .. | 3 | .. | . | . | 2 | . | . | 5 |
| Papilloma | . | .. | 1 | 1 | . | . | . | . | . | 2 |
| Embryonal Carcinoma | . | .. | 1 | .. | . | . | . | . | . | 1 |
| Totals | . | 13 | 7 | 18 | . | 9 | 6 | . | . | 53 |

| | |
|---|---|
| Total number of epithelial tumors | 53 |
| Total number of non-epithelial tumors | 89 |
| Total number of all tumors | 132 |
| Percentage of epithelial tumors | 40 |
| Number of malignant epithelial tumors | 46 |
| Number of non-malignant epithelial tumors | 7 |
| Percentage of epithelial tumors that were malignant | 86.8 |
| Percentage of epithelial tumors that were not malignant | 13.2 |
| Percentage of malignant epithelial tumors to total number of tumors received | 34.9 |

Malignant growths other than epithelial in nature are here classified.

| Kind of tumor | Mule | Horse | Dog | Bovine | Sheep | Swine | Fowl | Mouse | Rabbit | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Fibrosarcoma | 3 | 3 | 1 | 2 | . | . | . | . | . | 9 |
| Endothelioma | . | . | 1 | 1 | . | . | 1 | 2 | . | 5 |
| Hypernephroma | . | . | . | 4 | . | . | . | . | . | 4 |
| Mesothelioma | . | 1 | . | 1 | . | . | . | . | . | 2 |
| Melanosarcoma | . | 2 | 1 | . | . | 1 | . | . | . | 4 |
| Lymphosarcoma | . | . | 4 | 2 | 2 | 2 | 2 | . | 1 | 13 |
| Lymphocytoma | . | . | 1 | . | . | . | 9 | . | . | 10 |
| Totals | 3 | 6 | 8 | 10 | 2 | 3 | 12 | 2 | 1 | 47 |

Percentage of non-epithelial malignant tumors of total ..... 35.6

Malignant tumors of all varieties are grouped according to the following table.

| Kind of Tumor | Mule | Horse | Dog | Bovine | Sheep | Swine | Fowl | Mouse | Rabbit | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Carcinoma | . | 11 | 1 | 15 | . | . | 1 | . | . | 28 |
| Adenocarcinoma | . | 1 | 1 | 2 | . | 9 | 3 | . | . | 16 |
| Adamantinoma | . | 1 | . | .. | . | . | . | . | . | 1 |
| Embryonal Carcinoma | . | .. | 1 | .. | . | . | . | . | . | 1 |
| Hypernephroma | . | .. | . | 4 | . | . | . | . | . | 4 |
| Fibrosarcoma | 3 | 3 | 1 | 2 | . | . | . | . | . | 9 |
| Endothelioma | . | .. | 1 | 1 | . | . | 1 | 2 | . | 5 |
| Mesothelioma | . | 1 | . | 1 | . | . | . | . | . | 2 |
| Melanosarcoma | . | 2 | 1 | .. | . | 1 | . | . | . | 4 |
| Lymphosarcoma | . | .. | 4 | 2 | 2 | 2 | 2 | . | 1 | 13 |
| Lymphocytoma | . | .. | 1 | .. | . | . | 9 | . | . | 10 |
| Totals | 3 | 19 | 11 | 27 | 2 | 12 | 16 | 2 | 1 | 93 |

Percentage of total tumors that were malignant ..... 70.4

Non-malignant growths of all varieties were found as follows:

| Kind of tumor | Mule | Horse | Dog | Bovine | Sheep | Swine | Fowl | Mouse | Rabbit | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Fibroma | 3 | 5 | . | 5 | . | . | . | . | . | 13 |
| Lipoma | . | 1 | 1 | .. | . | . | . | . | . | 2 |
| Leiomyoma | . | . | . | 2 | . | . | 1 | . | . | 3 |
| Myxoma | . | . | . | 1 | . | 1 | . | . | . | 2 |
| Myxofibroma | . | . | 1 | .. | . | . | . | . | . | 1 |
| Melanoma | 1 | 2 | . | 4 | . | 2 | . | . | . | 9 |
| Lymphoma | . | . | . | 1 | . | . | . | . | . | 1 |
| Adenoma | . | . | 3 | .. | . | . | 2 | . | . | 5 |
| Papilloma | . | . | 1 | 1 | . | . | . | . | . | 2 |
| Cholesteatoma | . | 1 | . | .. | . | . | . | . | . | 1 |
| Totals | 4 | 9 | 6 | 14 | . | 3 | 3 | . | . | 39 |

Percentage of total tumors that were non-malignant ..... 29.6

*Points of Origin and Location.* A summary of the locations occupied by the 132 tumors shows that the following were involved one or more times. Eye, esophagus, lip, brain, jaw, concha, glans penis, leg, pectoral region, shoulder, neck, mediastium, lung, uterus, spleen, vagina, axillary space, lymph nodes, gizzard, oviduct, kidney, sublumbar region, hip, testicle, spinal canal, pharynx, prepuce, adrenal body, walls of thoracic cavity, below ear, region of flank, abdominal wall, poll, anus, thoracic cavity, mesentery, intestines, heart and nasal cavity.

The organs most frequently affected were the following:

| | |
|---|---|
| Kidney | 9 |
| Eye and its appendages | 20 |
| Lung | 6 |
| Liver | 8 |
| Spleen | 5 |
| Lymph nodes | 10 |
| Intestines | 4 |

The kidneys were most often affected in hogs, the spleen and liver in chickens and the eye in cattle and horses (twelve and seven times respectively). The lymph nodes were involved most frequently in the dog.

In many of the cases two or more organs were affected by the same tumor such as the lung and the lymph nodes, or the liver, spleen and kidneys.

The incidence of the disease was apparently not influenced by sex, tumors occurring with equal frequency in the male and female. In a few instances there were more females than males represented; this predominance occurred in the case of old cows of which there are a larger number slaughtered than old males.

The influence of color was not suggested except in the case of melanoblastomas. All the horses affected with this tumor were gray, while the cattle and swine affected were red.

The age incidence could not be satisfactorily determined for all groups of tumors because of the failure of the clinician to record the age in every case. The groups in which sufficient number of ages were given enables me to offer the following data.

AVERAGE AGE OF ANIMALS AFFECTED WITH THE FOLLOWING TUMORS

| | | |
|---|---|---|
| *Carcinoma:* | Horse | 10 years |
| | Bovine | 6 years |
| | Canine | 6 years |
| | Chicken | 2 years (one case) |
| *Lymphosarcoma:* | Canine | 6 years |
| | Sheep | 2 years |
| | Bovine | 7 years |
| *Fibrosarcoma:* | Horse | 8 years |
| | Bovine | 4 years |
| | Canine | 3 years (one case) |
| *Lymphocytoma:* | Chicken | 1 year |
| *Adenocarcinoma:* | Hog (kidney cases) | 2½ years |
| | Bovine | 4 years |
| | Avian | 2½ years |
| | Horse | 15 years (one case) |

The numbers of different kinds of tumors that occurred in the various species follow:

| | | | |
|---|---|---|---|
| Mule | 3 | Bovine | 13 |
| Horse | 10 | Sheep | 1 |
| Dog | 12 | Swine | 5 |
| | | Fowl | 7 |

The different species gave rise to the following number of tumors:

| | | | |
|---|---|---|---|
| Bovine | 41 | Mule | 7 |
| Horse | 28 | Sheep | 2 |
| Fowl | 19 | Mouse | 2 |
| Dog | 17 | Rabbit | 1 |
| Swine | 15 | | |

## DISCUSSION

From the available figures it is difficult to compare the frequency of tumors in the lower animals with the occurrence of neoplasms in man. That is, it is impossible, for instance, to say what per cent of the horse, dog or bovine population is affected with tumors at a certain age, while the information available does permit of such a statement as regards the human. The figures, as unsatisfactory as they are, do however suggest that there are probably but slight differences as regards the total incidence of tumors in the two classes (man and animals).

It is obvious that certain tumors appear with greater frequency than others and that there is quite a difference in the susceptibility of the various species towards neoplastic growth.

One cannot but be impressed by the large number of epithelial tumors encountered. In this series fifty-three cases or 40 per cent were of this variety and of these total malignancy was a feature in forty-six or 86.8 per cent. Malignancy seems to be the rule in the tumors of the lower animals as evidenced by the high percentage of malign growths in this study in which 70.4 per cent of all varieties possessed this feature.

The high incidence of malignant epithelial tumors is an interesting finding as is the fact that the epithelial malignancies practically equal the malignancies of all other kinds combined (forty-six epithelial malignant tumors and forty-seven non-epithelial malignant tumors).

A great many of the tumors such as the lipomas, mesotheliomas, lymphomas, myxomas and cholesteatomas were encountered but once or twice, which would suggest that these forms are comparatively rare in the lower animals. A few others such as chondroblastomas, osteoblastomas, rhabdomyoblastomas, neuroblastomas and glioblastomas, I was unable to add to my collection, and by failure to get material of these varieties from others, I am convinced that they must occur very infrequently.

The relative infrequency of neoplasms in sheep is another interesting point brought out by these figures. Only two of the 132 in the total were found in this animal. It is difficult to account for this apparent rarity on any other basis than a species insusceptibility. Of course, a great majority of sheep are slaughtered while lambs, and other than congenital neoplasms would have but little opportunity to develop during the short life of the animal. Again, however, a considerable number of old ewes come to postmortem examination annually, yet available data bear out the figures of my series and one must conclude that sheep are relatively immune to tumorous proliferations.

The mule is likewise peculiar in some respects. While the fibroblastomas commonly occur in this animal, other varieties must be rare. In the 132 cases reviewed, aside from the fibroblastomas, but one other tumor, a melanoma, was found. The racially close horse, on the other hand, was found to be particularly susceptible to the epithelial growths in addition to those affecting the mule. In comparison with all other species, the mule appears to be less subject to large numbers and varieties of tumors than any other domestic

animal, with the exception of the sheep. The horse, bovine and dog show the greatest susceptibility, with the bovine heading the list.

The most frequent tumors of the dog as they appeared in the above tabulations were the adenomas and the lymphosarcomas, while in the swine the familiar adenocarcinoma * of the kidney comes first. Aside from this one variety of epithelioblastoma, the hog is apparently infrequently affected with the epithelial tumors. In my cases no other types appeared.

The common fowl is likewise seldom affected with epithelial tumors and it is indeed rare that a true carcinoma is seen (but once in my series). By far the greatest number of chicken tumors belong to the lymphoblastomas. In my collection of nineteen neoplasms of the domestic fowl nine were lymphocytomas (leukemias) and two were lymphosarcomas. In no instance have I encountered neoplasms in the turkey, which seems unusual.

The bovine appears to be especially prone to tumors. In the cases previously listed forty-one tumors (about 31 per cent of the total) were secured from cattle and thirteen different types of tumors were represented in the total. In addition to the high tumor incidence in the bovine, this species seems susceptible to the greatest variety of new growths. The forty-one tumors listed as occurring in this animal consisted of thirteen different varieties. The dog is also interesting in this regard, the seventeen specimens from this species falling into twelve different groups, while the horse with twenty-eight tumors showed ten different kinds.

A large share of the epithelial malignant growths in the horse and bovine involved the eye and the appendages of that organ. Of the thirty-one epithelial growths affecting these two species, fifteen involved this organ with the disease apparently occurring most often in the bovine. The eye of the Hereford seemed to be particularly susceptible. The penis of the horse was also a common location occupied by carcinomas. Adenomas arise not uncommonly from the eye of the dog and the majority of the adenocarcinomas involved the kidneys of hogs (nine out of sixteen).

The distribution of the melanoblastomas is of some interest, being one in the mule, four in the horse, one in the dog, four in the bovine and two in the hog. Most workers have found this tumor far more

* I have used the term adenocarcinoma for that group of kidney tumors of swine commonly called adenosarcoma. A paper in support of this designation is in preparation.

frequent in the horse than in any other species. While these cases were scattered among the different species, I do not think that the figures should alter the accepted opinion in this regard. The scarcity of this tumor in the horse in my series is probably due to the comparatively few tumors in the total (132) and the fact that melanotic growths are so frequent in the horse that they were not considered of sufficient importance to be worthy of microscopic study. Again, melanomas are the easiest tumors to diagnose in the gross, and the curiosity which might have prompted the practitioner or meat inspector to send many other tumors to the laboratory for a diagnosis was absent in the case of these pigmented growths. The occurrence of this tumor in the other species suggests that perhaps the bovine, hog, mule and dog have more melanoblastomas than is ordinarily appreciated.

A tumor that is even more frequent than this report would indicate is the leukemic condition in chickens, more properly termed malignant lymphocytoma. In addition to those which appear in this work we have had a considerable number of these cases in our laboratory for diagnosis during the past few months. In fact this is without question the most frequent neoplasm with which chickens may be affected. Of 101 chickens passing through our laboratory during the past four months, eight, or 8 per cent, showed this condition.

It is not presumed that the figures offered in this report on the incidence of the various tumors in the different species are correct for the entire animal population represented. To the contrary, many of the percentages would probably be found decidedly erroneous if a sufficiently large number of specimens could be collected. They do, however, represent the approximate incidence in at least certain of the groups. This is more especially true with the epithelioblastoma and certain of the lymphoid tumors.

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# PRIMARY CARCINOMA OF THE LIVER OF POSSIBLE MULTICENTRIC ORIGIN OCCURRING IN A CASE OF PORTAL CIRRHOSIS *

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In a recent paper we presented a clinico-pathologic study of five cases of primary carcinoma of the liver occurring at the Mayo Clinic. As is well known, this rare tumor occupies a somewhat unique position, not only on account of its habits of growth and spread, but also by reason of its almost constant association with cirrhosis. Particular attention was paid to this relationship, both in the cases we reported and in those collected from the more recent literature. The incidence of cirrhosis, as given by the earlier writers, Eggel, Goldzieher and von Bókay, and Yamagiwa, varies from 70 to 80 per cent, especially for the hepatoma or primary liver cell form, but later reports indicate an even higher percentage and that cirrhosis is the main predisposing factor in the disease. Thus the cases reported by Karsner and by Winternitz, and nineteen occurring since 1922, all showed cirrhosis. The case of Helvestine is the only one in which it was absent.

Concerning the multicentric or unicentric origin of carcinoma of the liver there is considerable divergence of opinion. Winternitz, Karsner and others hold that the disease is unicentric and that its spread in the liver is metastatic by way of the portal and hepatic veins. Van Heukelom and Travis have described transitional forms at various points in the neighborhood of carcinomatous nodules and have concluded from this that the growth is multicentric. It is indeed on the basis of the presence or absence of these transitional forms that practically all writers have based their conclusions. It seems, therefore, that the question will never be satisfactorily settled so long as it depends on the individual interpretation of various stages of the carcinoma cell, a matter of extreme difficulty and varied interpretation under the best conditions. While our

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studies on this point led to nothing definite which might aid in the solution of the problem, our inability to find any such transitional forms forced us to agree with Winternitz and Karsner that in all probability the tumor was unicentric in origin. All of our cases were advanced, so that clear evidence of the earliest stages was difficult to obtain. However, the recent occurrence of a remarkably early case of primary carcinoma of the liver has caused us not only to reverse our previously expressed opinion but to bring forward evidence on a different basis, suggesting that the condition can be multicentric in origin as well as metastatic in spread.

### Report of a Case

A woman, aged 58 years, was admitted to the clinic complaining of stomach trouble. For the previous twelve years she had suffered attacks of epigastric pain with nausea and vomiting followed by considerable abdominal soreness. For the previous year the attacks had become so severe as to require sedatives, and were accompanied by edema of the feet and progressive swelling of the abdomen.

The patient was moderately jaundiced and had edema of the legs and feet and marked swelling of the abdomen due to fluid. The liver and spleen were not palpable. Large external hemorrhoids were present. The hemoglobin was 77 per cent. Erythrocytes numbered 4,090,000, and leucocytes 7,800 per cu. mm. The phenoltetrachlorphthalein test of hepatic function showed dye retention 3. Roentgenograms revealed multiple small stones in the gall bladder. A diagnosis of cholelithiasis with secondary cirrhosis of the liver was made. On exploratory operation the diagnosis was confirmed and a Talma-Morrison operation was performed. Paracentesis was carried out twenty-one days after operation but the patient gradually became weaker and died thirteen days later.

Necropsy showed portal cirrhosis of the liver with early primary carcinoma, ascites, bilateral hydrothorax and anasarca, dilatation of the veins of Sappey, of Retzius and of those of the esophagus, cholecystitis and cholelithiasis with dilatation of the gall bladder and ducts (Grade 2) and arteriosclerosis (Grade 3).

The skin and sclerotics were markedly jaundiced. The abdominal cavity contained 1,500 cc. of fluid while the pleural cavities together contained about the same amount. The subdiaphragmatic, intestinal and inferior hemorrhoidal veins were all markedly dilated and tortuous. The spleen weighed 209 gm. and presented patchy hyaline thickenings of the capsule with considerable fibrosis of the pulp. The gall bladder had thickened, opaque, white fibrous walls and contained 50 cc. of greenish black bile together with multiple, small, dark, faceted stones.

The liver weighed 846 gm. without the gall bladder. Its surface was yellowish white with a thick, tough, fibrous capsule covering a surface roughened all over by more or less coalescent nodules, from 2 to 4 mm. in diameter, giving the organ a finely granular appearance. On section it offered considerable resistance to the knife and presented a cut surface which was markedly granular, corresponding to the external appearance. Each of the small, rounded, yellowish areas of

hepatic tissue measured from 2 to 4 mm. in diameter, and was surrounded by a band of dense, glistening, fibrous tissue. In addition to this typically cirrhotic state there were three well defined, soft, greenish white nodules averaging 2 cm. in diameter and surrounded by dense connective tissue. From the capsules of each, a fine network of fibrous tissue radiated to the center, dividing the contents into smaller granules and supporting the rather pasty friable mass which constituted the nodules. Two of them were situated at the extremity of the left lobe while the other was in the right lateral portion of the right lobe. Gross serial sections showed that they could all be included in two slabs (Figs. 1 and 2) and that no other similar areas existed in any situation. From the photographs it can be seen that one piece (Fig. 1) included both nodules in the left lobe (*a* and *b*), and part of the nodule in the right lobe (*c*). The other included the remainder of the nodule (*c*) (Fig. 2).

## Pathologic Analysis

The surfaces of these slabs were then divided into definite numbered areas, and thin sections shaved from their whole extent for microscopic examination. Thus a series of large microscopic slides was prepared, each from a known area and representing the entire breadth of the liver in two planes. A careful search was made of all other organs for a possible primary focus. As none was found, the tumors were assumed to be primary in the liver rather than metastatic.

*Microscopic Examination.* Examination of the three nodules, *a*, *b* and *c*, shows them all to be of the same nature. Each is a partially encapsulated mass of cells quite irregular in arrangement and showing none of the characteristic radiate disposition of normal hepatic tissue. Nodule *a* consists of masses of polyhedral, triangular, round and oval cells varying greatly in size but very little larger than normal hepatic cells. For the most part, they have a definite trabecular arrangement forming anastomosing columns, with here and there many tubules which contain bile (Figs. 3 and 4). The cells are supported by a delicate stroma of connective tissue, more marked in some places than in others, although this is not a pronounced feature. Endothelium-lined spaces everywhere separate the cell columns; they are obviously vascular capillaries. The cells themselves are fairly easily distinguishable from the surrounding parenchyma of the liver, the nuclei being irregular, markedly hyperchromatic and basophilic, often multiple (Fig. 5), and exhibiting occasional mitotic figures (Figs. 6 and 7). The cytoplasm is more acidophilic than usual and is finely granular in appearance. Toward the center of the nodule degenerative changes are common, especially fatty vacuolation

of the cytoplasm, with nuclear pyknosis and karyolysis. Scattered throughout the capillaries are many polymorphonuclear and lymphocytic leucocytes.

Nodules *b* and *c* prove to be identical histologically with *a* except that fatty degeneration is much more prominent. The capsule of all three consists of dense fibrous tissue containing a few regenerating bile ducts and columns of hepatic cells. While a capsule is found around each nodule it does not in any way confine the tumorous tissue within its limits. Columns of malignant cells are found actively growing through and outside it, invading the parenchyma for a short distance on all sides (Fig. 8). It is evident that the capsules are condensations of the sclerotic tissue already present in the cirrhotic liver and that they are produced by the expansion of the carcinomatous tissue. A study of the tissue intervening between the nodules proves of the greatest interest. Scattered in the parenchyma between *a* and *b* are several masses of cells identical with those in the larger nodules. While some appear to be lying free among the hepatic cells, others are obviously within endothelium-lined spaces or vascular channels. It seems therefore that these are metastatic growths and represent the path of extension either from *b* to *a*, or *vice versa*. With the exception of this area and of that just outside the capsule of the nodules, a close and careful examination of all the other slides, particularly from the area between *b* and *c*, fails to reveal any sign of definitely malignant cells.

The cirrhotic areas themselves were next examined. An extremely severe chronic hepatitis is in progress with diffuse infiltration of the liver columns by masses of lymphocytes. These are for the most part collected in and around the dense fibrous tissue which surrounds the nodules of the liver parenchyma, although a more diffuse and intercellular type of infiltration is not uncommon. A general view of any one of these nodules shows it to consist of irregularly anastomosing columns of double liver cells, devoid of any normal or lobular arrangement with regard to the central vein. In no area can these central veins be distinguished, although endothelium-lined spaces in the surrounding fibrous tissue indicate that they have been engulfed by it in the constant destructive and constructive processes at work. Each nodule shows considerable fatty degeneration, bile stasis with bile thrombi and venous congestion.

It was to the individual cell, however, that more particular atten-

tion was directed. Where the hepatitis is more diffuse, regenerative and hyperplastic forms are evenly distributed throughout the nodules, along with the débris of cells in the process of dissolution. Generally speaking, this regeneration is more marked at the periphery than at the center, where atrophic forms are much more numerous. The chief distinguishing points in these newly formed cells are their size, shape, staining reactions and characteristic nuclei. They tend to be larger and more convex, with clear cut edges and more acidophilic cytoplasm than the normal cell. The normal nuclei are usually fairly uniform in size and are composed of a moderately basophilic lacework of chromatin, often containing a single nucleolus. In any field on the slides the eye is immediately attracted to these hyperplastic cells by reason of the larger and more irregular nuclei, and by their more basophilic and hyperchromatic tendencies. Although mitotic figures are infrequent, binucleate, trinucleate and multinucleate forms are relatively common. Between the single and binucleate cells, dumb-bell forms are occasionally noted, while much larger cells, containing as many as nine nuclei, are also found. These multinucleated forms occur in practically every regenerating nodule in the cirrhotic areas, yet none can be definitely classed as malignant.

## Discussion

It is important, in the first place, to distinguish between the condition we have described and the so-called nodular hyperplasia or adenomatous regeneration of the liver. The term hepatoma, later appropriated by Yamagiwa for the liver cell type of carcinoma, was originally used by Sabourin for this condition. He considered it to be an intermediate stage between cirrhosis and carcinoma, a view held by many subsequent writers. According to Mallory it is found more frequently in those acute toxic types of cirrhosis in which degeneration and regeneration are so rapid that excessive new growth of liver cells results in the formation of greenish yellow, encapsulated nodules 1 cm. or more in diameter. Aschoff, Rolleston and Ribbert emphasize the difficulty in making a gross diagnosis between the two conditions, and Hansemann states that adenoma may pass into carcinoma with no definite line of demarcation. Ribbert, indeed, holds that the liver cell type of carcinoma almost invariably represents malignant change within these adenomatous masses.

Well marked examples are, however, distinguished microscopically by the degree of differentiation of the cells and by their non-invasive characteristics. While the cirrhosis in this case was of an extremely chronic type, the cells of the nodules showed so little differentiation, and appeared to possess such infiltrative power that the diagnosis was undoubtedly carcinoma.

The two points of great interest with regard to primary carcinoma of the liver which still require elucidation are its multicentric or unicentric origin and its relation to cirrhosis.

With regard to the first point the following evidence may aid in its solution. In an extremely cirrhotic liver three nodules were discovered which were proved histologically to consist of primary carcinoma of the hepatoma or liver cell type. Two of them (*a* and *b*) were situated in the left lobe; microscopic examination of the intervening tissue disclosed carcinomatous masses filling the vascular channels. It is therefore probable that either *a* metastasized from *b*, or that each appeared independently of the other. The occurrence of nodule *c* at a distant point in the right lobe sheds additional light on the problem. Both *b* and *c* were practically of the same size. Except for the areas immediately surrounding the capsules, complete microscopic examination of the cirrhotic tissue between *b* and *c* revealed no definite carcinomatous tissue or any tumor thrombi in the intervening vascular or lymphatic channels. The usual route by which these carcinomas spread is by either the portal or hepatic veins, but both these channels were quite free.

How then did the nodules *b* and *c* reach their respective positions? At this point it is important to bring forward an anatomic fact about the liver which has been demonstrated in another connection (McIndoe). From a vascular or biliary point of view the liver is a symmetrical organ with right and left lobes definitely supplied by the right and left branches of the portal vein and hepatic artery, and drained by the two branches of the bile duct. The line of demarcation runs through the middle of the gall bladder fossa to the point of entrance of the hepatic veins into the inferior vena cava. This has been proved to be constant for a large series of normal livers injected through the separate branches, and it bears no relationship to the usually described arrangement of five lobes (Figs. 9 and 10). Not only the afferent vascular channels but the bile ducts participate in this bilateral arrangement, so that the liver may be

said to consist essentially of two more or less equal halves with distinct vascular supply and biliary drainage. No vessels larger than capillaries cross the boundary line separating the two lobes except in the case of the hepatic artery, where there is a slight arteriolar anastomosis from side to side. It has also been shown that the lymphatics lie along the distribution of portal spaces and the hepatic veins, and that the flow is efferent in both cases (Helvestine, Lee).

The nodules *a* and *b* can be seen to lie well within the left half of the liver, while *c* lies in the right half. By microscopic examination, tumor thrombosis and embolism were found between *a* and *b*, but none between *b* and *c*. Direct extension may therefore be excluded. The chances of embolism from side to side are remote, for not only must the embolus have traversed the capillaries at the line of demarcation, but it must have done so against the vascular flow in all three channels including the lymphatics. The wide separation of the nodules almost precludes the chance of spread by way of the slight arteriolar anastomosis. The possibility of an embolus being swept into the inferior vena cava and through the general circulation until it finally lodged in the right lobe, or *vice versa*, is also very remote. As a general rule, with these tumors extension is direct within the liver and metastasis to distant organs uncommon even in the most advanced cases. The alternative probability is therefore that the nodules in the left lobe arose independently of those in the right, thus making them multicentric in origin.

Concerning the relationship of the cirrhosis to carcinoma little need be said. For Sabourin and the majority of subsequent writers, carcinoma follows in the footsteps of previous cirrhosis; for Hanot and Gilbert, cancer and cirrhosis develop simultaneously under the influence of the same irritating agent; and for Lancereaux and Wegelin, the cirrhotic lesions are secondary to the cancer. Here there can be but little doubt that the cirrhosis with its twelve-year history preceded the onset of carcinomatous change, a fact confirmed by the chronicity of the inflammatory reaction and the large amount of dense fibrous tissue found histologically. Moreover, all of the cirrhotic areas showed the most extreme hyperplastic changes, sometimes generalized and sometimes peripheral. Nuclear division has been so rapid that as many as nine nuclei were found in one hepatic cell. They were also more basophilic and hyperchromatic

than usual, tending to be larger and to exhibit one or more nucleoli with a distinctness and irregularity which are regarded by Broders as evidence of more marked hyperplasia and possibly precancerous change. Although, as has been frequently stated, the interpretation of multiple nodules of hyperplastic cells, as indicating multiple foci of carcinomatous change, should be made guardedly, yet the occurrence of this alteration in areas bearing such an anatomic relationship to each other makes the possibility somewhat stronger. It seems probable, therefore, that the carcinoma was superimposed on the extreme hyperplastic change resulting from severe generalized chronic hepatitis.

## Summary and Conclusions

A case of long-standing atrophic cirrhosis of the liver has been described, in which early carcinomatous change of the hepatoma or liver cell type had taken place, and in which death was the result of the cirrhosis. The carcinoma was a secondary change. Two carcinomatous nodules were found in the right and left lobes of the liver, respectively, with no evidence of a common thrombotic or embolic origin. They were in areas of independent vascular supply and lymphatic drainage. In the absence of any possible channel permitting direct or embolic extension, these nodules are believed to have arisen independently of each other, and to represent a carcinoma of multicentric origin.

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## DESCRIPTION OF PLATES

### PLATE 103

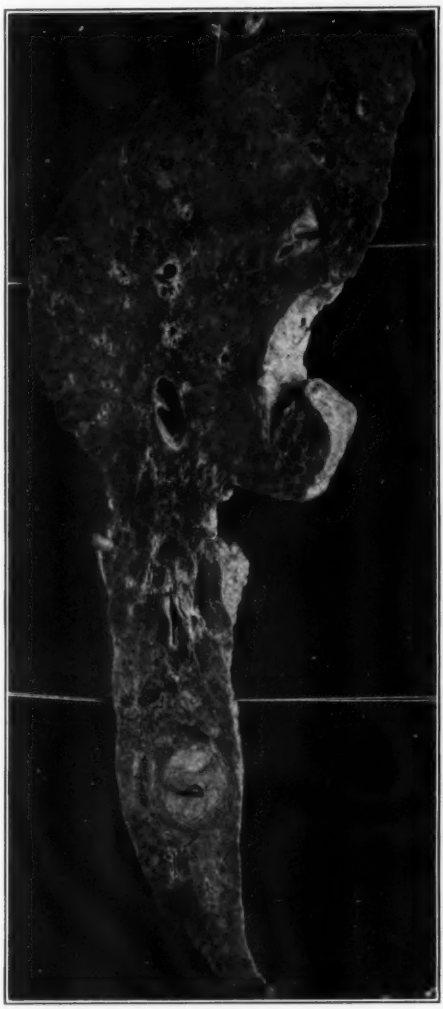
FIG. 1. Longitudinal section of liver showing marked atrophic portal cirrhosis with the nodules *a*, *b* and *c* distributed in the right and left lobes.
FIG. 2. Section of liver showing the remainder of nodule *c*.

### PLATE 104

FIG. 3. Bile capillaries in tumor distended with fluid and inspissated bile. × 250.
FIG. 4. Masses of tumor cells showing tubule formation. Note capillary network and absence of stroma. × 300.
FIG. 5. A multinucleated tumor cell. × 1000.
FIG. 6. A mitotic figure from nodule *b*. × 1000.
FIG. 7. A mitotic figure from nodule *c*. × 1000.
FIG. 8. Edge of nodule *c*, showing liver cells on right side and tumor cells on left. Direct invasion of parenchyma without the intervention of any capsule. × 250.

### PLATE 105

FIG. 9. Corrosion specimen of the two branches of the portal vein to show the independence of the two sides.
FIG. 10. Corrosion specimen of the two branches of the hepatic artery to show the definitely bilateral blood supply. Line of demarcation corresponds to the portal vein.



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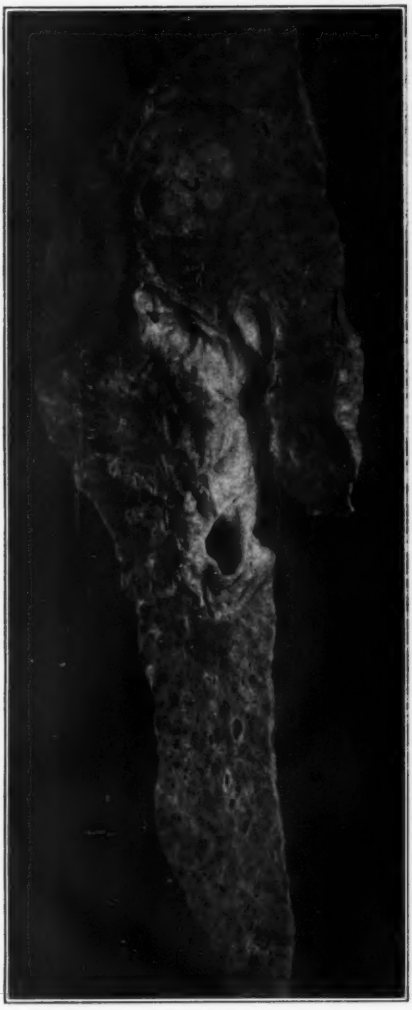

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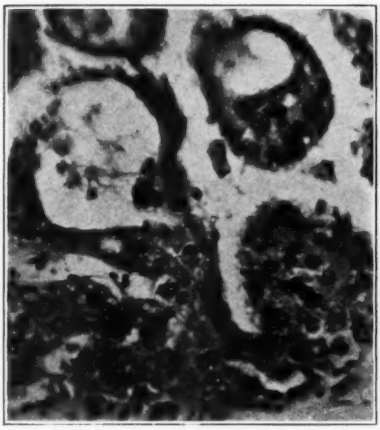
McIndoe and Counseller

Primary Carcinoma of the Liver



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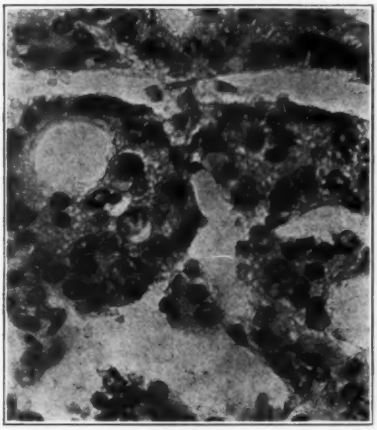

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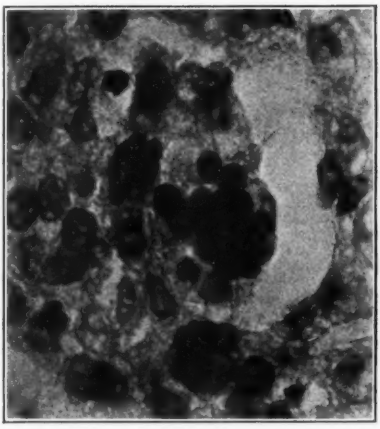

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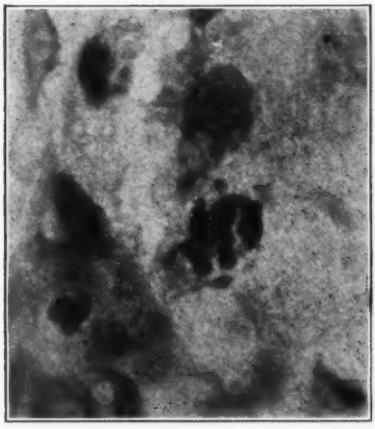

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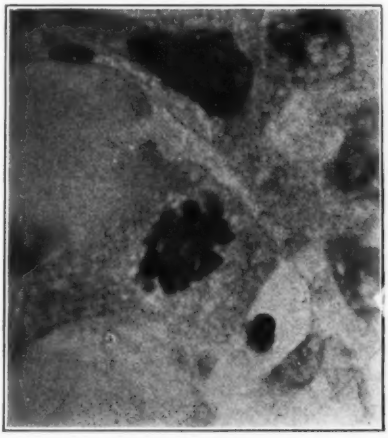

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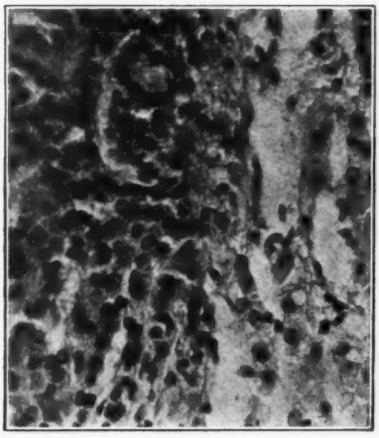

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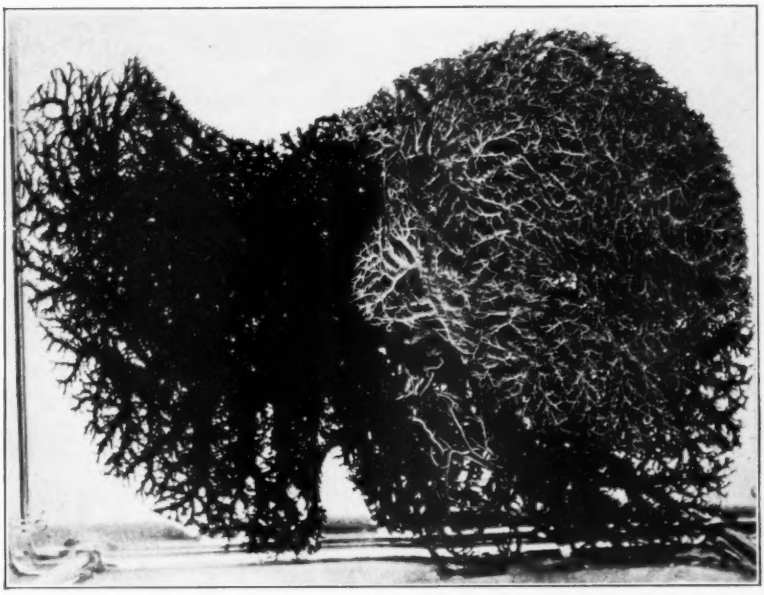
McIndoe and Counseller

Primary Carcinoma of the Liver



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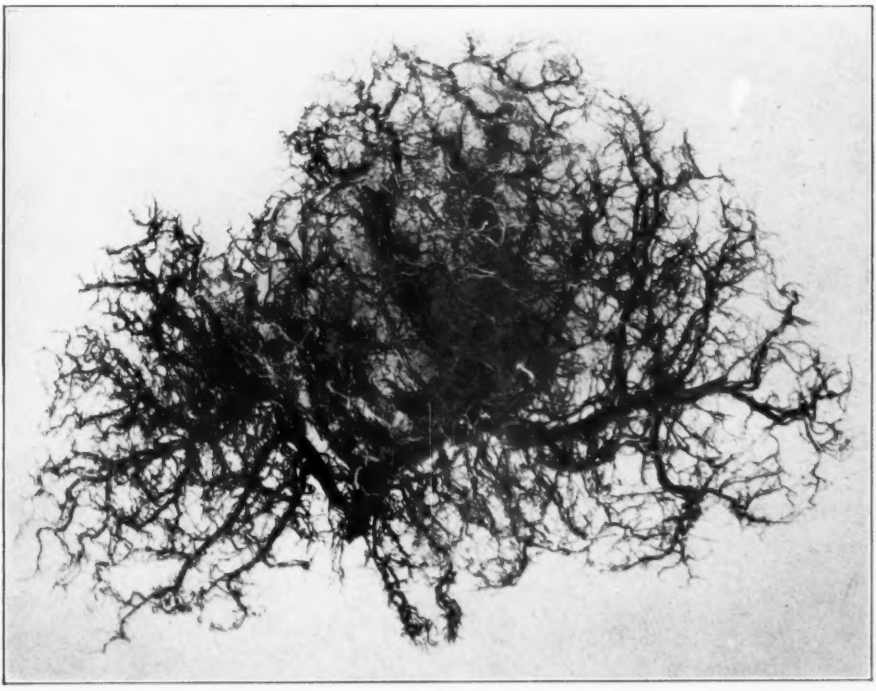

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McIndoe and Counseller Primary Carcinoma of the Liver

# STUDIES ON THE VASA VASORUM *

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Some observations have been made during the course of an investigation concerning the vasa vasorum, which seem to be of sufficient interest to report. Briefly stated, the experimental procedure consisted in the injection of the vessels of different animals with India ink gelatin. It was found possible to demonstrate the vasa vasorum by injecting the gelatin into the lumen of the vessel. More satisfactory preparations were obtained, however, by injecting the vasa directly through specially devised glass cannulae. Specimens were rendered transparent by Spalteholtz' method. The immediate presentation has to do with observations made on the aorta of the horse and the dog.

In the aorta of the horse, the vasa vasorum are found to penetrate through the media to the intima, where they terminate abruptly. Fig. 1 represents a transverse section from a portion of the aorta of the horse, with one of the larger vasa dipping down from the adventitia toward the intima. The section illustrates how the finer capillaries tend to spread out in a direction parallel to the circular muscle fibers and elastic tissue plaques, between which they run. Fig. 2 is an enlargement of the preceding picture and shows one of the branches spreading out into its finer arborizations. A longitudinal strip from the same aorta (Fig. 3) shows the abrupt line marking off the vascular media from the avascular intima. In Fig. 4 a diffuse network of vessels is seen in the adventitia of this aorta, with arterioles and venules running side by side.

In the aorta of the dog there are three outstanding considerations of interest. Vasa vasorum have been observed to arise directly from the lumen of the ascending aorta and supply this portion of the vessel. Fig. 5 shows one such vessel injected directly through the

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The data presented in this communication are taken from the dissertation presented to the Faculty of the Yale University School of Medicine in partial fulfillment of the requirements for the Degree of Doctor of Medicine.

small opening in the aorta at *a*, and also an extensive anastomosis including a branch *b* anastomosing with one of the coronary arteries.

That these openings occur normally in the aorta of the dog is indicated by the fact that the examination of the aortas of twenty-one animals revealed their presence in all but one. The aorta in this instance was observed to be the seat of extensive sclerotic change. The number of openings varied markedly, one aorta showing seven, while in several aortas only one opening was present. The commonest number was five, present in six of the aortas. Whether or not the absence of openings, mentioned in the one instance, is of significance in its association with the sclerotic change remains for further investigation to elucidate.

The commonest origin of the vasa vasorum of the aorta is from the branches of that vessel. Arising usually a few millimeters from the mouth of the branch, they run back to spread out on the surface of the larger vessel. This is shown in Fig. 6 which represents an aorta injected through its lumen, after each intercostal artery had been tied off, then opened and laid flat during the clearing process. The dark spots represent the intercostal vessels, the vasa vasorum running back from them to the aorta. The same arrangement is true with regard to the coronary arteries which give off vasa to the base of the aorta.

In addition to the above, a third point of interest was found in the highly vascularized pads of fat at the base of the dog's heart. The pads frequently assume the most bizarre shapes and appear to fill in every crevice between the auricles and ventricles and the surrounding pericardium. Frequently one of the discrete openings of the vasa is found in the aorta immediately underneath one of the fat pads. When injected, the pads fairly balloon out, because of the filling of the vascular net which permeates them. See Figs. 7, 8 and 9.

## Summary

1. Illustrations are given showing the vasa vasorum in the aorta of the horse extending through the media to the intima.

2. Vasa vasorum are pictured arising from the intercostal arteries of the aorta of the dog.

3. An illustration showing a vas vasis arising directly from a discrete opening in the ascending portion of the aorta of the dog is given.

4. Fat pads at the base of the aorta of the dog are described and illustrated.

5. Data are given regarding the occurrence of discrete openings of the vasa vasorum in the aorta of the normal dog and the question is raised whether the absence of these openings is significantly associated with sclerotic change in the aorta.

## DESCRIPTION OF PLATES

### PLATE 106

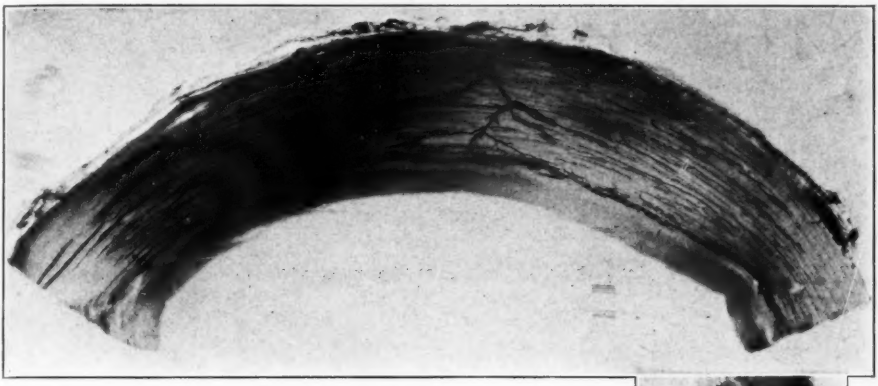
FIG. 1. Transverse section, horse's aorta. × 4.
FIG. 2. Transverse section, horse's aorta. × 10.
FIG. 3. Longitudinal strip, horse's aorta. × 3.

### PLATE 107

FIG. 4. Adventitial vessels, horse's aorta. × 10.
FIG. 5. Discrete opening of vasa vasorum in ascending portion of dog's aorta. × 2.
FIG. 6. Vasa vasorum arising from intercostal arteries, dog's aorta. × 2.

### PLATE 108

FIG. 7. Transverse section from ascending portion of dog's aorta, showing fat pads. × 3½.
FIG. 8. Fat pad on ascending portion of dog's aorta; pulmonary artery at *P*. × 3½.
FIG. 9. Enlargement of tip of fat pad; epicardial covering at *e*. × 12.



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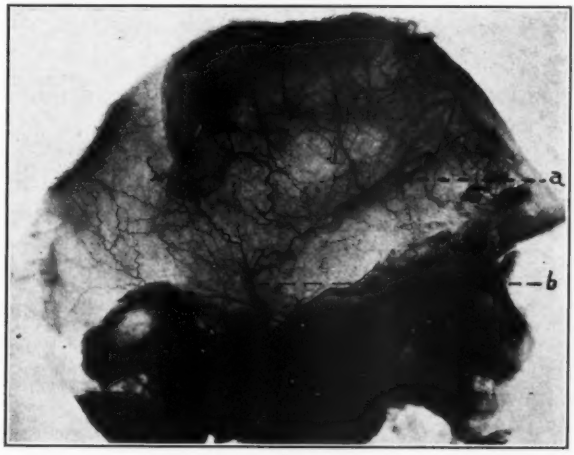


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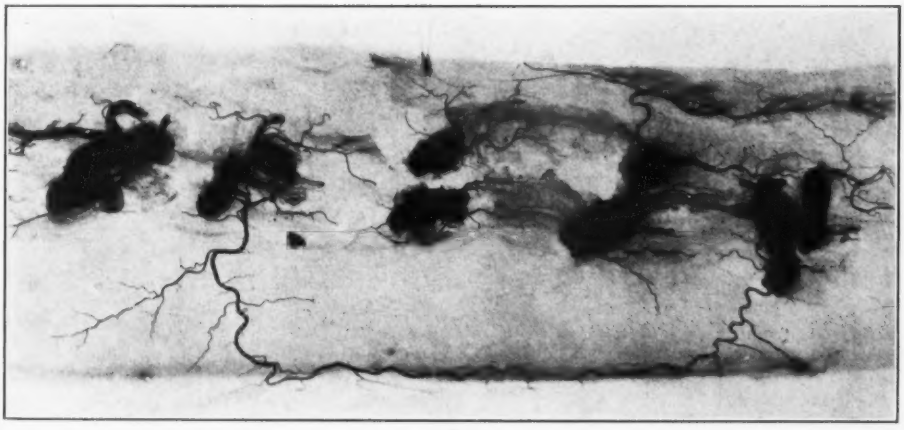

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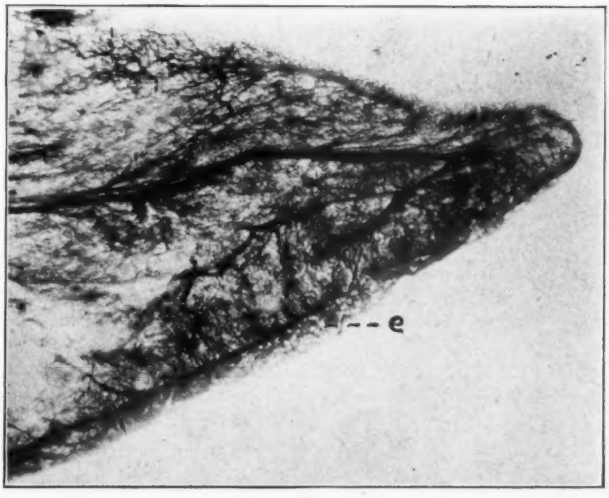


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# THE SIGNIFICANCE OF GIANT CELLS IN THE INTRADERMAL TUBERCULIN REACTION *

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Numerous writers in studying the intradermal tuberculin reaction in tuberculous guinea-pigs have called attention to the curious phenomenon usually designated as the "lighting-up of a previous site of reaction." From time to time in retesting a tuberculous animal either intracutaneously or by other routes, an intradermal tuberculin reaction obtained several days previously may be observed to show an increase in surrounding erythema. No very satisfactory explanation of this interesting phenomenon has as yet been offered. Coca [1] presents a theory which may be briefly summarized as follows: The character of the histologic changes that take place at the site of the local tuberculin reaction is not without significance with respect to its anaphylactic nature. It appears that in some of these lesions that were subjected to microscopic study, the changes resembled those of tuberculosis while in others they could not be distinguished from the typical lesions of that disease. The pathology of the local tuberculin reaction appears, thus, to be that of tuberculosis, and the specific character of this lesion may have some relation to the instances of a "lighting-up" at the sites of previous injections after a subsequent one. Such a change is conceivably due to a mechanism similar to that which underlies the "focal reaction."

In support of his contention Coca quotes the observations of Bandler and Kreibich,[2] and Daels.[3] Reference to this first paper, however, reveals the fact that these authors did not regard their histologic findings as indicative of a local tubercle formation. They state quite clearly that the giant cells and epithelioid cells seen by them differ from the Langhans type of cell and they note especially their prevalence in fat-containing tissues. Daels, however, did consider the reaction to be of the nature of a local tubercle and believed it due to the presence of occasional killed bacilli and fragments of

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bacilli in the tuberculin. An attempted differentiation between the Langhans cell and the foreign body giant cell may be wholly superfluous since Medlar [4] sees in the giant cell of tuberculosis nothing more than a foreign body giant cell. He states that giant cells are an indication of a reparative process in small areas of caseation or of simple necrosis of tissue — a reaction to a foreign body. This contention would appear to vary considerably from the concepts recently enunciated by Cunningham, Sabin and their co-workers,[5] these latter believing the tubercle to be a highly specific structure, indicative of a peculiar symbiosis between the tubercle bacillus and the monocyte.

The authors, in an attempt to study the question of tuberculin desensitization in guinea-pigs had occasion to examine histologically a number of intradermal tuberculin reactions. The usual giant cells were observed and it became desirable to analyze the factors responsible for their formation. Ten per cent old tuberculin was used to elicit the reaction and injections were made intracutaneously, enough tuberculin being used to produce a bleb 6 or 7 mm. in diameter. Tissues were available for study at hourly stages from the ninth to the twenty-third hour and at 1, 2, 3, 4 and 6 days.

The early stages of the reaction may be passed over briefly. They are characterized by marked edema, fibrin deposit, exudate of polymorphonuclear leucocytes and eosinophiles and necrosis of certain tissues. The necrosis is dependent upon the intensity and depth of the reaction. Necrosis of epithelium and fat cells occurs with great regularity. In intense reactions one sees necrosis of collagen; in deeper reactions some necrosis of muscle with invasion by polymorphonuclear leucocytes occurs. The subsequent course of events is best described by reference to the accompanying photomicrographs. Fig. 1, taken from an early skin test, shows a group of fat cells; about two of these cells is a subsiding reaction; immediately outside the clear area of fat is a narrow zone of polymorphonuclear leucocytes, the cells which surrounded the fat during the stage of acute reaction. About these cells is a second zone consisting of endothelial leucocytes attracted by necrotic fat. Fig. 2 shows a slightly later stage (four days); here essentially the same condition is found but the endothelial leucocytes surrounding fat and polymorphonuclear leucocytes have fused to form small foreign body giant cells; in addition to these small giant cells there are two large

giant cells, one of which contains fat droplets and partially surrounds a fat cell. Numerous fibroblasts are present. Gradually polymorphonuclear leucocytes disappear and the resultant picture is that of giant cells surrounding fat or containing globules of fat (Figs. 3 and 4). Still later all traces of fat may vanish leaving giant cells embedded in dense fibrous stroma (Fig. 5).

Foreign body giant cells may form about other structures than fat. We have observed them to a very limited extent about old fibrin and necrotic collagen. Necrosis of fat cells, however, is the chief exciting agent in their formation.

If such an etiology is correct, then non-specific necrosing agents may be expected to produce a similar result and this is in fact the case. Mallein in full strength (250 mg. per cc.) produces in the skin of normal guinea-pigs a reaction grossly similar to the intracutaneous tuberculin reaction and in its later stages the microscopic picture in every way resembles that of the tuberculin reaction. Giant cells are formed in identical fashion. Consequently, there is nothing specific in the formation of these giant cells *per se*. That giant cells may form following necrosis of tissue in actual tuberculous lesions, is an undoubted fact, but there too Medlar has justly doubted any specific character. We likewise would regard them as non-specific structures developing in response to necrosis, and see no reason for drawing immunologic conclusions from their presence.

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## DESCRIPTION OF PLATES

### PLATE 109

FIG. 1. Early intradermal tuberculin reaction; a subsiding acute inflammatory reaction about fat cells. × 500.

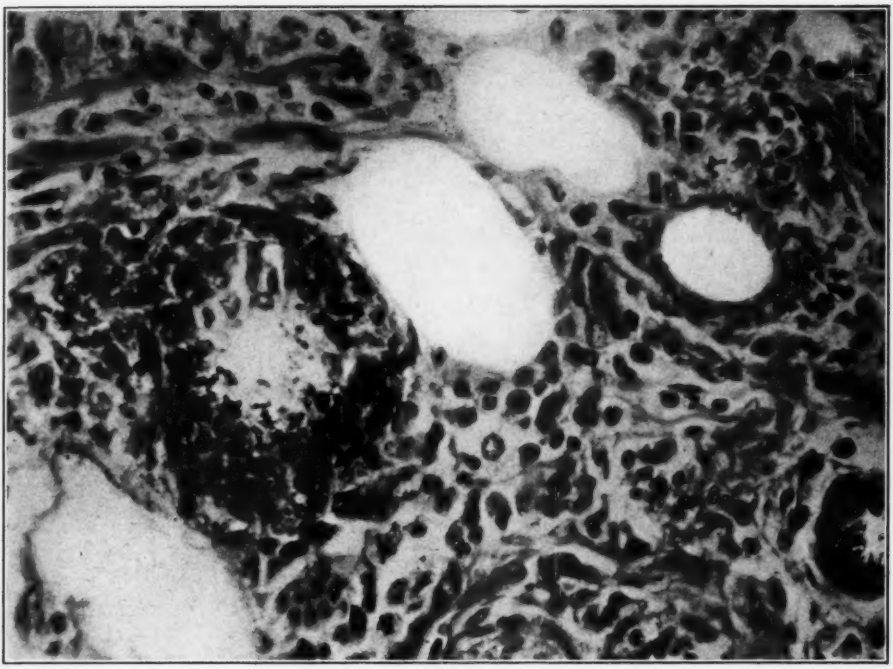
FIG. 2. Further subsidence of reaction, four day skin test. Giant cell formation about fat. × 500.

### PLATE 110

FIG. 3. Large giant cells about fat; same animal as Fig. 2. × 500.

FIG. 4. Numerous fat globules in giant cells, four day skin test. × 500.

FIG. 5. Older giant cells remaining in dense scar tissue on the sixth day. × 250.



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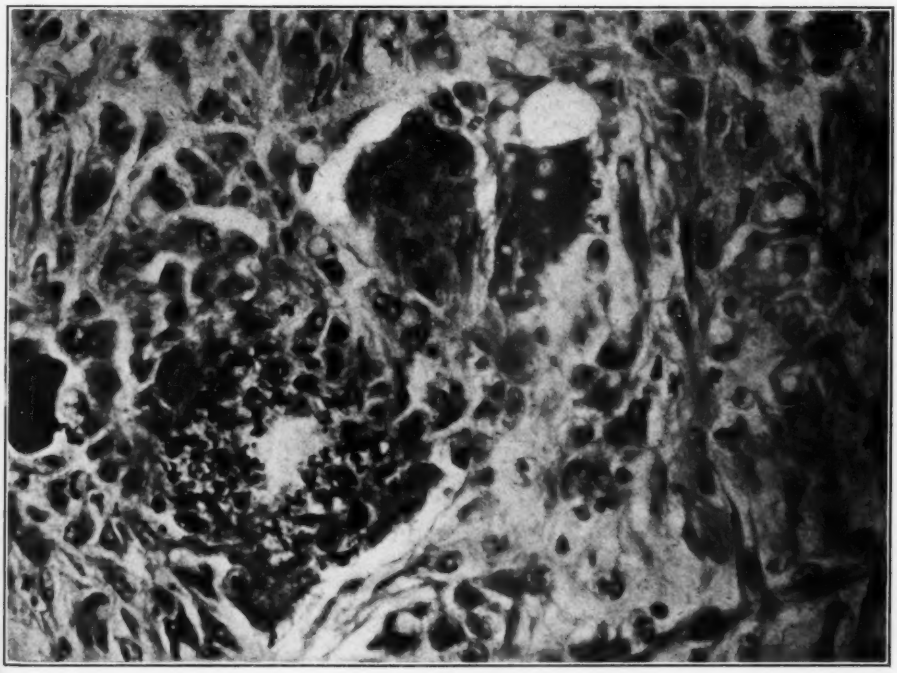

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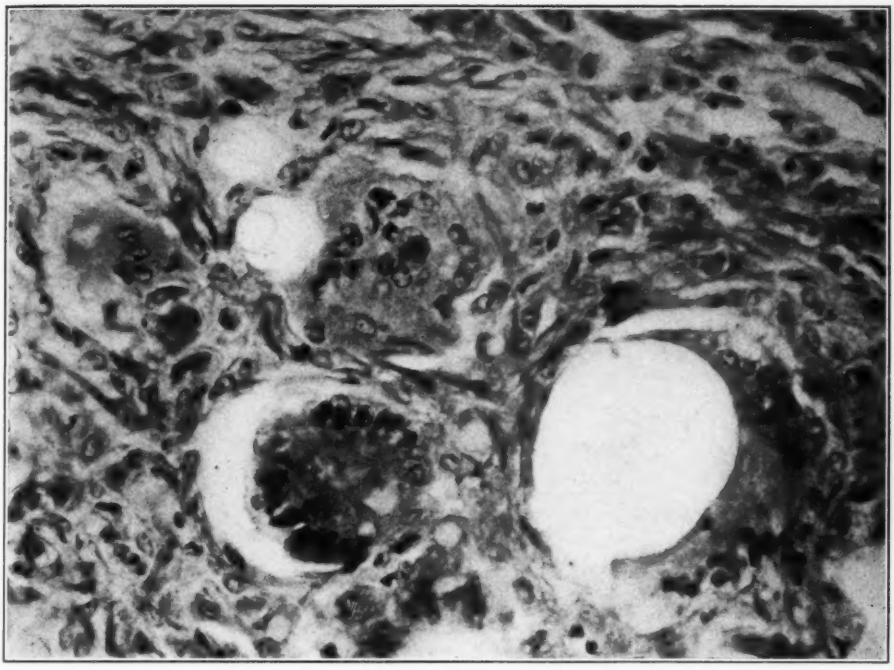
Stewart and Rhoads Giant Cells in the Tuberculin Reaction



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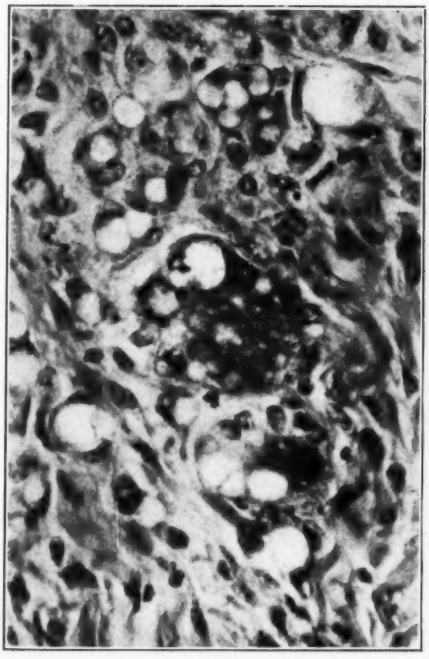

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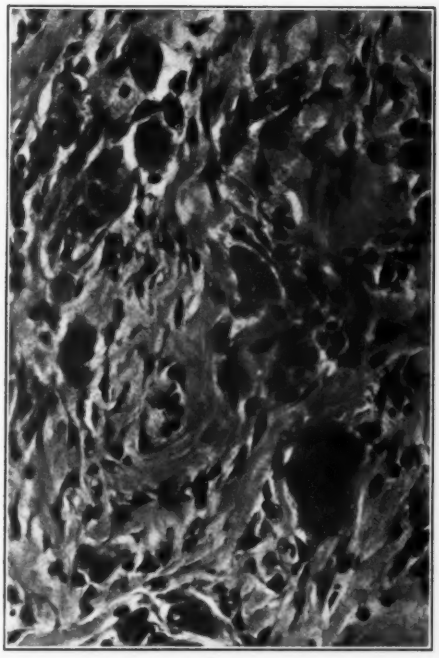

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Stewart and Rhoads

Giant Cells in the Tuberculin Reaction

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* Abstract of paper presented at the meeting of the Association of Pathologists and Bacteriologists held at Albany, N. Y., April 2 and 3, 1926.

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